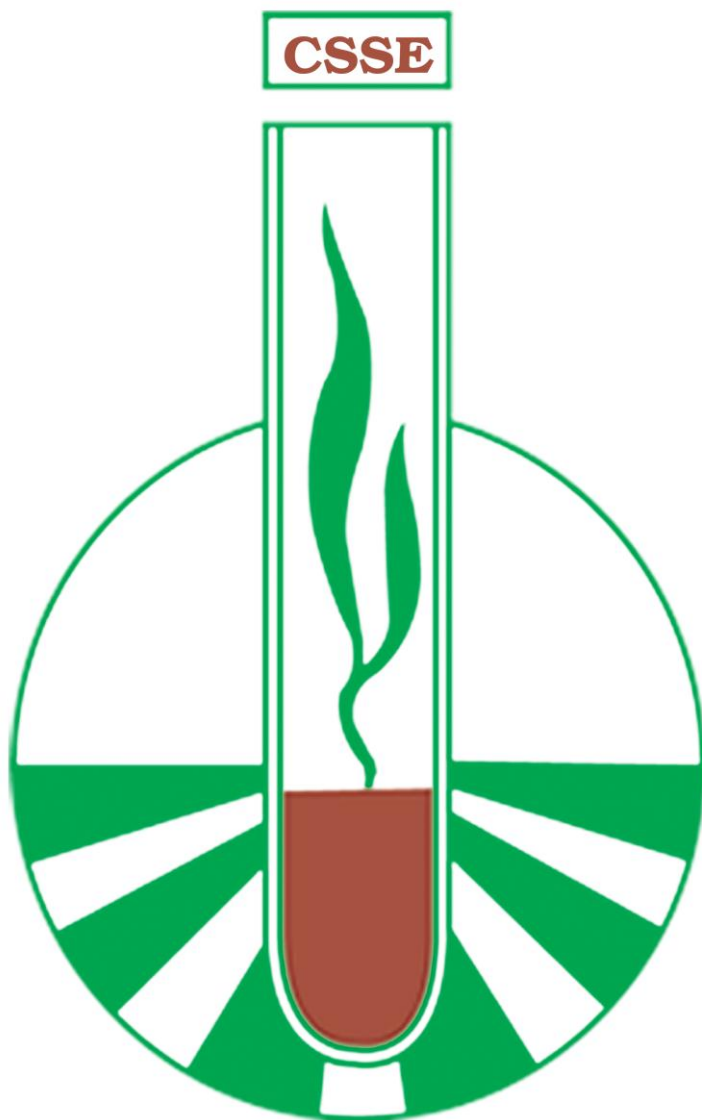


# Ethiopian Journal of Crop Science

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# Grapevine Inflorescence and *Botrytis cinerea*: An Attempt to Understand the Molecular Queues during Initial Infection and Entrance into Quiescence

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## Abstract

**Botrytis cinerea**, a cosmopolitan necrotrophic fungus, is the cause of gray mold in a wide range of crops including grapevine, where it affects both fruit quality and yield. The pathogen is difficult to manage due to the quiescent state of infection ensuing after the primary infection. Primary infection mostly occurs at bloom by airborne conidia; and the fungus remains quiescent until maturity and egresses at ripe to causes bunch rot. To understand the molecular crosstalk between the grapevine and **B. cinerea** during initial infection and entrance into quiescence, inflorescences of **Vitis vinifera** (cv. Pinot Noir) were inoculated with **B. cinerea** at anthesis. Infections were halted at 12, 24, 48, 72 and 96 hours post inoculation (hpi) and samples were subjected to confocal microscopy, quantitative polymerase chain reaction and RNA-sequence analyses. It was observed that most of the conidia germinated and form apressoria-like structures within 24 hpi, which resulted in penetration of the flower epidermis. During penetration, **B. cinerea** increased the expression of its genes encoding for virulence factors that instigated defense responses from the flowers side. The defense responses involved genes associated with the accumulation of pathogenesis-related proteins, stilbenoids, reactive oxygen species and cell wall reinforcement. At 96 hpi the transcriptional reaction appeared largely diminished both in the host and in the pathogen, implying **B. cinerea** became quiescent, forced by the defense responses of the host, until conditions favor egression.

**Keywords:** *Botrytis cinerea*; defense response; grapevine; inflorescence; quiescence.

## Introduction

Grape vines (*Vitis* spp.) are among the most worldwide-grown perennial fruit crops with a global production of more than 80 million tons (FAOSTAT, 2017). Major economic benefits come

from wine and related fermented products, but also from fresh fruit, raisins, fruit juices and jams. However, its production is jeopardized by adverse environmental conditions and biotic threats. The crop is prone to several diseases and pests, and their management consumes a large

proportion of its cultivation cost (Creasy and Creasy, 2009). Diseases from phytopathogenic fungi and oomycetes like *Plasmopara viticola*, *Erysiphe necator*, and *Botrytis cinerea* are the major cause of damage in quality and yield of grape.

*Botrytis cinerea*, a cosmopolitan necrotrophic fungus, causes pre- and post-harvest diseases in a wide range of crops including grapevine. The fungus can live pathogenically but also saprophytically or endophytically (van Kan et al., 2014; Shaw et al., 2016). Besides the different mode of infections the pathogen has, it propagates by mycelia and/or conidia, and survives for extended periods as sclerotia (Williamson et al., 2007). During infection, *B. cinerea* induces necrosis by producing toxins and reactive oxygen species (ROS) (van Kan, 2006), and manipulates host metabolism to facilitate colonization (Williamson et al., 2007). *Botrytis* can quiescently infect immature fruits where that damage is mostly exhibited during ripening. Host factors that derive the pathogen into quiescent are proposed to be the presence of preformed and inducible antifungal compounds, inactivation of fungal pathogenicity factors, and firm berry skin (Prusky et al., 2013). Thickness and permeability of grape berry cuticle and its epicuticular waxes, the biochemical composition and morphology of the berry skin, and ripening stage are described as important berry skin features regarding the resistance/susceptibility of berries to *B. cinerea* (Deytieux-Belleau et al.,

2009; Becker and Knoche, 2012; Herzog et al., 2015).

In vineyards, *B. cinerea* is part of the natural microflora where previous-season infection is the main source of primary infection (Nair et al., 1995). *Botrytis* infection is possible to occur at any stage of berry development, but primary infection often occurs during bloom (McClellan and Hewitt, 1973; Nair et al., 1995; Keller et al., 2003). A mechanistic model based study suggested that infection status on inflorescences and young clusters explain well *B. cinerea* epidemics in vineyards (González-Domínguez et al., 2015). Higher disease severity, at harvest, was observed from grapevine inflorescences received *Botrytis* inoculation at full bloom, (Keller et al., 2003). After the initial infection at bloom, the pathogen generally remains quiescent until the onset of fruit ripening and it resumes active growth thereafter to cause bunch rot (McClellan and Hewitt 1973; Keller et al., 2003). Thus, bunch rot observed at maturity may not only be due to *de novo* infection of mature berries, it rather could be from the latent infection established earlier at bloom.

Studies so far conducted on the quiescent infections of *B. cinerea* in grapevine inflorescences (for example: McClellan and Hewitt, 1973; Nair et al., 1995; Keller et al., 2003) demonstrated that flower infection is an important stage in the epidemiology of *B. cinerea* in grapevine. The intriguing phenomena that forces *B. cinerea* stay quiescent until grape

berry ripening remained are not fully understood. Though reprogramming of carbohydrate and lipid metabolisms towards increased synthesis of secondary metabolites involved in plant defense, such as resveratrol phytoalexin, was observed during incompatible interaction of *B. cinerea* and unripe grape berries (Agudelo-Romero *et al.*, 2015; Kelloniemi *et al.*, 2015). Thus, the crosstalk between *B. cinerea* and grapevine during initial infection at bloom remains as a subject for research, and therefore this study aimed at understanding the molecular queues entailed at initial infection and entrance to quiescence.

## Materials and Methods

### Plant material and *B. cinerea* inoculation

Fruiting cuttings of cv. Pinot Noir, collected from an experimental vineyard of the Fondazione Edmund Mach, were used to raise inflorescences. Cuttings with 3-4 nodes were induced to root by treating with 300 ppm of indol-3-butyric acid for 15 min. After five to six weeks, individual rooted cuttings were repotted into a 1.5 l pot using commercial potting mix. Following budburst, the vegetative apex was removed to promote inflorescence growth. *B. cinerea* (isolate B05.10) was incubated on potato dextrose agar (PDA) on plate at 25 °C. Conidia were harvested with distilled water from 10 days old plate, and concentration was adjusted to  $2 \times 10^5$  conidia ml<sup>-1</sup> using

hemacytometer. At full cap-fall stage, each flower received 1.5 µl of a conidial suspension or distilled water (mock inoculation) closer to receptacle area. Inoculated cuttings were immediately bagged by water sprayed, clear plastic bag for 24 h to create high humidity. A transformant strain of B05.10, expressing green fluorescent protein (GFP) was used for microscopic observation and for post-inoculation evaluation of flowers/fruitlets.

### Microscopic observation and detection of quiescent *B. cinerea*

Thin slices of fertilized gynoecia were cut for confocal microscope observation about the state of *B. cinerea*. While for detecting the presence of quiescent *B. cinerea* in the first 14 days post inoculation, 8 fruitlets from each of six biological replicates were plated out on PDA (with Hygromycin B at 70 µg ml<sup>-1</sup>), before or after washing, for a week at room temperature. Washing was made with sterile water, a 1 minute three rinses with gentle shaking. An inflorescence from a fruiting cutting was considered as a biological replicate.

### RNA extraction, quantitative polymerase chain reaction (qPCR) and RNA-sequence analyses

Samples were collected at 12, 24, 48, 72 and 96 hpi in three biological replicates and snap frozen in liquid nitrogen, and kept at -80 °C until use.

RNA was extracted using Plant Total RNA Kit following the manufacturer's protocol. For qPCR assays, cDNA was synthesized from 3 µg of RNA, using SuperScript™VILO™cDNA

Synthesis Kit. qPCR was performed in a Viiia7 thermocycler using 0.31 µl of cDNA and 2.5 µM of primers in a total volume of 12.5 µl, where half of the total volume was Fast SYBR Green Master Mix. The reference genes used were *VvActin* and *VvTub*, and *Bcrpl5*

and *BcTubA*, for grapevine and *B. cinerea*, respectively. All primers and corresponding gene identifiers are provided in Table 1. Expression level of a gene was determined relative to its expression level in mock inoculated samples or PDB cultured *B. cinerea*. Statistical significance was calculated by Tukey's Honestly Significant Difference test or unpaired heteroscedastic Student's *t* test.

Table 1. Genes name and primers sequence used in quantitative polymerase chain reaction

| Gene name       | Forward and Reverse primer sequences                   |
|-----------------|--------------------------------------------------------|
| <i>BcACTA</i>   | CGTCACTACCTTCAACTCCATC; CGGAGATACCTGGGTACATAGT         |
| <i>BcBOA6</i>   | CAGCAATCGTTGTCCTGAAATC; GTTATCGCGTTCTTCACCTGTTA        |
| <i>BcCUTA</i>   | TGCTGGCAGTCAGACTATGG; TTCGGCTGGTAAAAGTTTGG             |
| <i>BcTUBA</i>   | TTTGGAGCCAGGTACCATGG; GTCGGGACGGAAGAGTTGAC             |
| <i>BcβGLUC</i>  | TGCAGCTACCTTTGATCGTG; TCCTTCCCAGTTACGTCCAC             |
| <i>Vv4CL</i>    | TTCCCGACATCAACATCCCG; TTACGTGCGGTGAGATGGAC             |
| <i>VvACT</i>    | ATGTGCCGTGCCATGTATGTTGCC; AGCTGCTCTTTGCAGTTTCCAGC      |
| <i>VvCAD</i>    | GTGGAGGTGGGATCAGATGT; TCCATCTCTGATTGTCATGG             |
| <i>VvCCR</i>    | AGCAGAAACAGGGATGCCAT; AGAGAGCCTCCCCTCTGACA             |
| <i>VvMYB14</i>  | TCTGAGGCCGGATATCAAAC; GGGACGCATCAAGAGAGTGT             |
| <i>VvPR10.1</i> | GCACATCCCGATGCCATTAAAG; ACTTACTGAGACTGATAGATGCAATGAATA |
| <i>VvPR10.3</i> | GAAATCCTACAAGGACAGGGAGGT; CGGCCTTGGTGTGGTACTTTT        |
| <i>VvST29</i>   | GGTTTTGGACCAGGCTTGACT; GAGATAAATACCTTACTCTATTCAAC      |
| <i>VvST41</i>   | GAGTACTATTTGGTTTGGACCT; AACTCCTATTTGATACAAAACACGT      |
| <i>VvTUB</i>    | TGTTGGTGAAGGCATGGAGG; AGATGACACGCCTGCTGAAC             |
| <i>VvWRKY33</i> | ATTCAAGCACTAGTATGAACAGAGCAG; CCTTGTGCCTTGGCATGA        |

Samples from 24 and 96 hpi were subjected for RNA-seq analysis a Next Generation Sequencing Platform HiSeq 1500 (Illumina, San Diego, CA). Genome assemblies of grapevine (Grape genome 12Xv1, <http://genomes.tribi.unipd.it/>) and *B. cinerea* (strain B05.10) (EnsembleFungi, <http://fungi.ensembl.org>) were used as reference sequences. The alignment was made by Subread aligner and raw read counts were extracted using the feature Count read summarization program.

Genes were considered differentially expressed if they had fold change of  $\geq 1.5$  and  $p$ -value  $< 0.05$ . Gene ontology enrichment was computed using customized annotation and annotated reference of GO terms into the AgriGO analysis tool (<http://bioinfo.cau.edu.cn/agriGO/analysis.php>; Du Z *et al.* 2010) and enriched GO terms (FDR  $< 0.05$ ) were visualized using the 'Reduce + Visualize Gene Ontology' (REViGO) webserver (<http://revigo.irb.hr>; Supek *et al.*, 2011).

## Results and Discussion

### *Botrytis cinerea* infection of grapevine inflorescence

Visualization of inoculated flowers at 24 hpi (Figure 1A) with confocal microscopy revealed that within 24 hours fungal conidia germinate, develop appressoria, and penetrate into the flower cuticle, on gynoecium above the floral disc (Figure 1B). Despite this, there was no subsequent visible progress in the infection

process and fruitlets look macroscopically healthy (Figure 1C-D). However, the plating out activity carried out on these healthy looking fruitlets showed that about 90% of them, either washed or not, carried *B. cinerea* that they received at cap-off stage (Figure 1E). The presence of the pathogen without visible symptom or sign for such a longer period may suggest that the fungus was not actively growing to cause disease, entered into quiescent phase after penetrating the flowers cuticle.

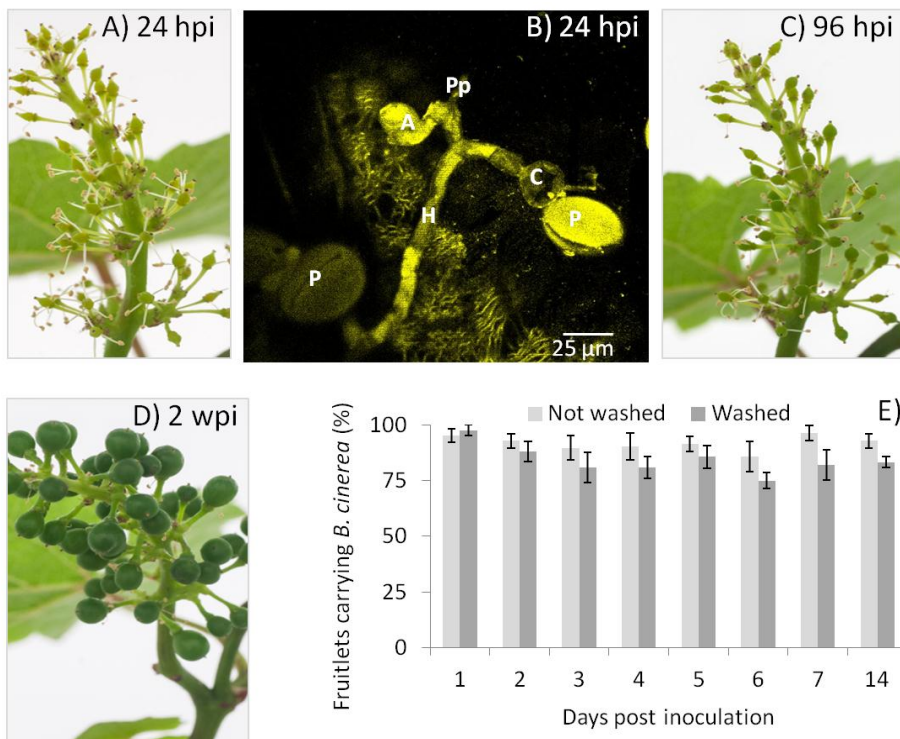


Figure 1. *Botrytis cinerea* infected grapevine flowers. Flowers inoculated with GFP-labelled B05.10 at full cap-fall stage (A) at 24 hours post inoculation (hpi), (B) confocal microscope image showing the fungus infecting flower cuticle at 24 hpi (C) asymptomatic fruitlets at 96 hpi and (D) 2 weeks post inoculation (WPI). A, appressoria; C, conidium; H, hypha; P, pollen grain; Pp, penetration peg. (E) Plating out of infected fruitlets on selective media (PDA with Hygromycin B, 70 µg/ml) before (NW) or after washing (W). Values at each day represent mean proportion of fruitlets showing GFP-labelled B05.10 growth on the selective media. Error bars indicate standard error. One way ANOVA computed on square root transformed data showed that mean proportions of W and NW, of each day, was not significantly different ( $P \geq 0.173$ ). Also mean proportions throughout the two weeks within W or NW was not significantly different ( $P \geq 0.160$ ).

## Sequencing the infected flowers transcriptomes

The biological variability within replication and among experimental conditions was assessed using hierarchical clustering, based on Spearman's correlation. The results indicated that there is a good degree of correlation within biological replicates of each experimental condition at 24 hpi than 96 hpi (Figure 2A). From the hierarchical clustering, two distinct clusters were formed by 24 and 96 hpi, irrespective of treatment conditions, except for "Ctrl 3-96" and "Trt 1-24"

(Figure 2A). The latter one has higher correlation value to the other two biological replicates of its kind than the rest of all. In the first cluster, which was comprised by samples from 24 hpi, the two inoculated samples were closer to each other than mock inoculated ones. However, in the second cluster, comprised by samples from 96 hpi, there were no such distinct differences among *B. cinerea* and mock inoculated samples, suggesting that samples at 96 hpi were similar at a whole transcriptome level.

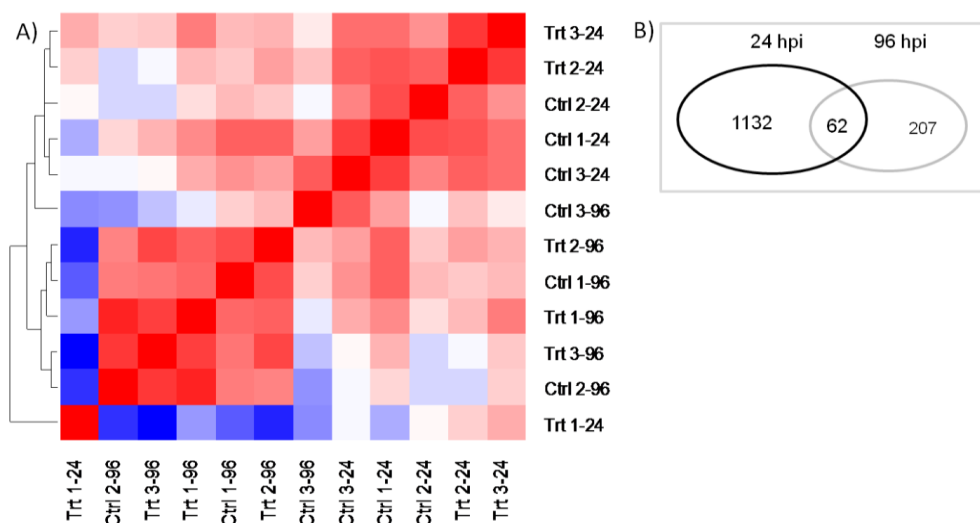


Figure 2. Global evaluation of the RNA-seq experiment and total differentially expressed (DE) genes. (A) Heat map of Spearman's correlation of the 12 samples. Ctrl, mock inoculated; Trt, *B. cinerea* inoculated; Bc, *Botrytis cinerea*; 1-3 indicates the biological replicates; and 24 and 96 are hours post inoculation (hpi). (B) Numbers of differentially expressed genes ( $P < 0.05$ , fold change  $> 1.5$ ) up on *Botrytis cinerea* infection at 24 and 96 hpi. Differential expression of grapevine genes was computed from the total expressed genes (20,530 out of the total 29,970 *V. vinifera* genes) in the inflorescences. Of these expressed genes, 1401 of them were differentially expressed (DE): 1194 DE genes at 24 hpi, whereas only 265 DE genes at 96 hpi (Figure 2B). The common DE genes between the two time points was limited to 62. Interestingly, at 24 hpi, the plant seems to respond to the presence of the pathogen with a prevalent induction of genes, which appeared to be no longer modulated at 96 hpi.

### ***Botrytis cinerea* transcripts detected in planta during grape flower infection**

According to the RNA-seq analysis, 836 *Botrytis* genes had raw reads of more than 10, and this set of genes was referred as *in planta* detected genes (Figure 3A). Of these 836 genes, 818 and 287 were detected at 24 and 96 hpi, respectively; 269 genes being common both for 24 and 96 hpi (Figure 3B). GO slim analyses were performed on the 836 *in planta* detected genes. Among the most represented GO terms in biological processes were related to translation, carbohydrate metabolism, energy metabolism, and anabolic and catabolic processes (Figure 3B), which are suggestive for cell wall degradation being activated and thereby initiation of infection. In the case of molecular functions ontology, functions related to ion binding and oxidoreductase activity were represented highly, indicating a widespread reprogramming of fungal transcriptome for pathogenesis. For example, oxidoreductase activity, a functional class represented most at 24 hpi than 96 hpi (Figure 3B), comprises genes involved in ROS production and scavenging activities, which is an important *B. cineria*'s pathogenesis mechanism. Overall, the molecular functions associated to the *in planta* detected *Botrytis* genes were

consistent with the functional categories in biological processes. In the case of cellular components ontology, since there were a lot of translational activities, as expected, "Ribosome" and "Protein complex" were among the most represented ones (Figure 3B). Furthermore, overrepresentation analysis of the *in planta* detected genes, based on annotated *B. cinerea* genes (Amselam *et al.*, 2011; Blanco-Ulate *et al.*, 2014; Espino *et al.*, 2010; Schumacher *et al.*, 2014), showed that genes encoding for proteins of early secretome, enzymes acting on plant cell wall (mainly on hemicellulose and pectin), and generation and deactivation of ROS were overrepresented (Table 2).

As depicted in Figure 4, genes involve in *Botrytis* virulence were upregulated more at 24 than 96 hpi. The higher expression of *B. cinerea* cutinase (*BcCUTA*) at 24 hpi implies the breaching of cuticle layer by appressoria. Similar expression profile was also observed by another cell wall degrading enzyme beta-glucosidase (*BcβGLUC*), degrading both cellulose and hemicelluloses. Botcinic acid (*BcBOA6*) gene encodes a polyketide synthase, one of the key enzymes in the biosynthesis of phytotoxic secondary metabolites.

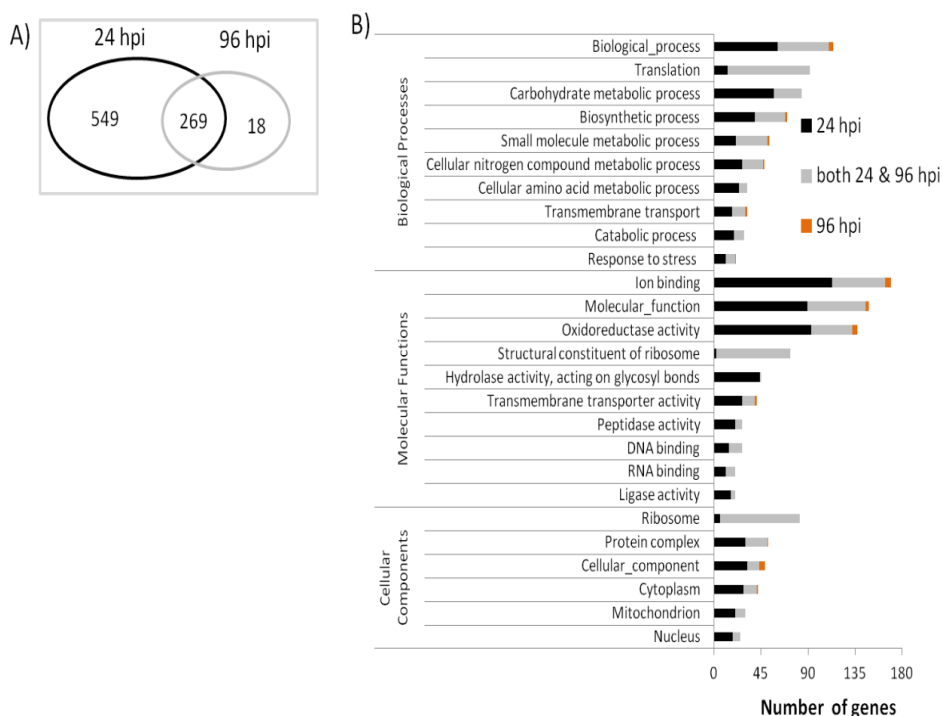


Figure 3. Number of detected *Botrytis cinerea* genes and their associated GO terms. (A) Venn diagram showing the number of detected genes unique or common to 24 and 96 hpi. (B) GO slim terms of *B. cinerea* genes detected *in planta*.

For *B. cinerea*, breaching plant cell wall is a key process to establish infection (van Kan, 2006; Williamson *et al.*, 2007; Blanco-Ulate *et al.*, 2014). After penetrating the cuticle, when infection is successful, the fungus grows into a pectin-rich epidermal cell (van Kan, 2006; Williamson *et al.*, 2007) by deploying its pectinases. Within 24 hpi, genes encoding for enzymes involve in cell wall depolymerisation were observed enriched and expressed significantly (Table 2 and Figure 4). Despite the observed readiness of the fungus to infect the grapevine flowers, there was no visible *Botrytis* symptom observed though the presence of quiescent *B. cinerea* was confirmed (Figure 1E). This implies that, the pathogen could not able to grow actively due to the

fierce defense it faced from the flowers. According to Prusky (2013), quiescence of a pathogen can happen at different developmental stages like before or after conidia germination / appressoria formation, and/or at subcuticular hyphae stage. Quiescent infections can occur in cellular locations like cuticular wax, intercellular space, and epidermal cells (Prusky *et al.*, 2013). In unripe tomato, *Colletotrichum gloeosporioides* was reported to enter into quiescent as a swollen hyphae after its appressorium germinated in to the cuticular wax (Alkan *et al.*, 2015). With regard to *B. cinerea* in grapevine, hyphae in the epidermal cell of immature berries were supposed to stay quiescence (Keller *et al.*, 2003).

Table 2. Functional categories of *Botrytis cinerea* genes detected in the inoculated grapevine flowers

| Functions of selected <i>Botrytis cinerea</i> genes                                          | Total no. of genes | 24hpi              |    |          |            | 96hpi              |    |          |            |
|----------------------------------------------------------------------------------------------|--------------------|--------------------|----|----------|------------|--------------------|----|----------|------------|
|                                                                                              |                    | no. detected genes | of | pvalue   | odds ratio | no. detected genes | of | pvalue   | odds ratio |
| Total number of predicted genes (B05.10 strains) (Amselem et al 2011)                        | 16448              | 818                |    |          |            | 287                |    |          |            |
| Proteins identified as early secretome, within 16 h of germination (Espino et al., 2010)     | 102                | 34                 |    | 0.00E+00 | 6.7        | 7                  |    | 2.30E-02 | 3.93       |
| Carbohydrate-Active Enzymes (CAZymes) (Blanco-Ulate et al 2014)                              | 1155               | 150                |    | 0.00E+00 | 2.6        | 28                 |    | 8.50E-02 | 1.39       |
| CAZymes acting on fungal cell wall                                                           | 166                | 26                 |    | 0.00E+00 | 3.1        | 4                  |    | 2.90E-01 | 1.38       |
| CAZymes acting on Plant Cell Wall                                                            | 151                | 51                 |    | 0.00E+00 | 6.8        | 5                  |    | 1.44E-01 | 1.90       |
| CAZymes acting on cellulose                                                                  | 10                 | 3                  |    | 7.50E-02 | 6.0        | 0                  |    | 9.99E-01 | 0.00       |
| CAZymes acting on hemicellulose                                                              | 58                 | 18                 |    | 0.00E+00 | 6.2        | 2                  |    | 2.43E-01 | 1.98       |
| CAZymes acting on hemicellulose and pectin side chains                                       | 28                 | 7                  |    | 0.017    | 5.0        | 0                  |    | 1.00E+00 | 0.00       |
| CAZymes acting on pectin                                                                     | 55                 | 18                 |    | 0.00E+00 | 6.6        | 1                  |    | 4.84E-01 | 1.04       |
| Proteins generating Reactive Oxygen Species (ROS) (Schumacher et al., 2014)                  | 53                 | 8                  |    | 2.90E-02 | 3.0        | 1                  |    | 4.70E-01 | 1.08       |
| Proteins involved in the detoxification of ROS (Schumacher et al., 2014)                     | 98                 | 20                 |    | 0.00E+00 | 4.1        | 5                  |    | 6.90E-02 | 2.92       |
| Protease (Amselem et al 2011)                                                                | 377                | 30                 |    | 2.00E-02 | 1.6        | 5                  |    | 7.59E-01 | 0.76       |
| Secondary metabolism key enzymes (Amselem et al 2011)                                        | 42                 | 2                  |    | 0.524    | 1.0        | 0                  |    | 1.00E+00 | 0.00       |
| 60S & 40S ribosomal protein (Amselem et al 2011)                                             | 81                 | 77                 |    | 0.00E+00 | 19.1       | 77                 |    | 0.00E+00 | 54.48      |
| Appressorium-associated genes (orthologs in <i>Magnaporthe oryzae</i> ) (Amselem et al 2011) | 12                 | 6                  |    | 1.40E-02 | 10.1       | 2                  |    | 1.02E-01 | 9.55       |
| Transporters (Amselem et al 2011)                                                            | 442                | 52                 |    | 0.00E+00 | 2.4        | 17                 |    | 1.00E-02 | 2.20       |
| Transcription factors (Amselem et al 2011)                                                   | 410                | 19                 |    | 6.23E-01 | 0.9        | 4                  |    | 9.41E-01 | 0.56       |

Significance was calculated using Fisher's Exact test. P values (<0.05) and odds ratios higher than 2 are highlighted

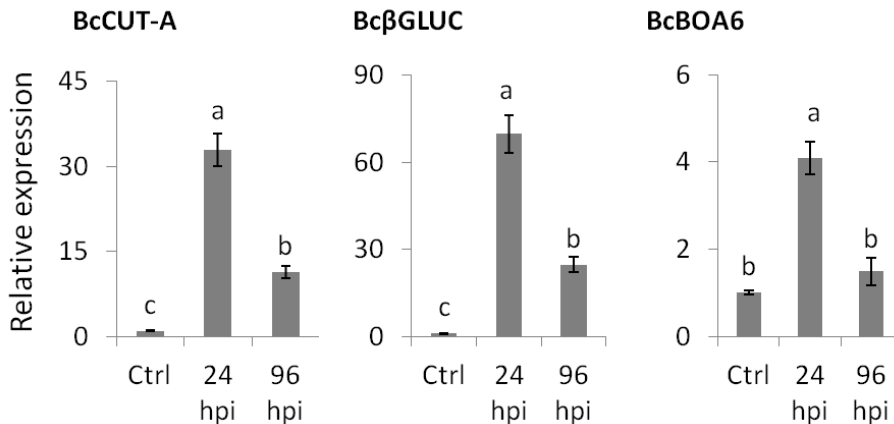


Figure 4. Expression profile of virulence-related *Botrytis cinerea* genes during grapevine flower infection [at 24 and 96 hours post inoculation (hpi)] relative to PDB cultured *B. cinerea*. BcCUTA (*B. cinerea* cutinase A), BcβGLUC (*B. cinerea* beta-glucosidase), BcBOA (*B. cinerea* botcinic acid), and ctrl (PDB cultured *B. cinerea*). Error bar is the standard error of the mean of three biological replicates. Expression values followed by a common letter are significantly not different among ctrl, 24, and 96 hpi samples, according to Tukey's Honestly Significant Difference test ( $P \leq 0.05$ ), using one way ANOVA.

From the microscopic and plating out activities of this study, *B. cinerea* entered into quiescent state after penetrating the first few epidermal cell layers, although quiescence as ungerminated conidia could also be possible.

All together, from the microscopic and transcriptomic results, the fungus attempted to infect grapevine flowers before entering into quiescence. Appressoria germination into flower gynoecium (Figure 1B), enrichment of functional categories required for causing disease such as plant cell wall degrading enzymes and ROS (Table 2) and differential regulation of virulence-related genes (Figure 4) were indications for the readiness of the pathogen to establish infection. In addition to these facts, most of the *in planta* detected *Botrytis* genes were also found highly expressed during successful infection of lettuce (De

Cremer *et al.*, 2013), tomato (Smith *et al.*, 2014), and ripe grapevine berry (Kelloniemi *et al.*, 2015).

### Transcriptional alterations of grapevine inflorescence upon *B. cinerea* infection

Gene ontology (GO) term enrichment analyses were made to evaluate the biological processes, molecular functions, and cellular components affected most due to *B. cinerea* infection (Table 3). At 24 hpi, significantly enriched ( $FDR < 0.05$ ) biological processes include many defense responses, for example, responses to biotic stimulus, biosynthesis of secondary metabolites, and protein modification process. In accordance to this defense related activities, signal transduction and kinase activities, carbohydrate binding, and transferase activity were also significantly overrepresented GO terms of molecular functions. From the

RNA-seq analysis, all sorts of genes that take part during biotic stress were differentially regulated. In the signaling pathway, among others, wall-associated kinases and phytohormones were observed to

involve at the initial stage of host-pathogen interaction (Table 4). Different genes of cell wall and secondary metabolism were also induced.

Table 3. Gene ontology terms enriched in the differentially expressed grapevine genes upon *B. cinerea* infection. Enriched GO terms at 24 and 96 hours post inoculation are presented.

| HPI | GO term    | Ontology | Description                  | FDR      |
|-----|------------|----------|------------------------------|----------|
| 24  | GO:0019748 | BP       | secondary metabolic process  | 2.60E-32 |
| 24  | GO:0009607 | BP       | response to biotic stimulus  | 6.90E-08 |
| 24  | GO:0009875 | BP       | pollen-pistil interaction    | 1.70E-04 |
| 24  | GO:0006464 | BP       | protein modification process | 8.70E-05 |
| 24  | GO:0007049 | BP       | cell cycle                   | 1.30E-04 |
| 24  | GO:0009056 | BP       | catabolic process            | 8.80E-04 |
| 24  | GO:0003774 | MF       | motor activity               | 4.40E-09 |
| 24  | GO:0030246 | MF       | carbohydrate binding         | 1.50E-04 |
| 24  | GO:0016301 | MF       | kinase activity              | 9.50E-03 |
| 24  | GO:0004871 | MF       | signal transducer activity   | 5.80E-03 |
| 24  | GO:0016740 | MF       | transferase activity         | 6.80E-03 |
| 24  | GO:0005576 | CC       | extracellular region         | 5.60E-04 |
| 96  | GO:0005576 | CC       | extracellular region         | 1.80E-06 |
| 96  | GO:0005618 | CC       | cell wall                    | 8.10E-05 |

NB: BP, biological process; MF, molecular function; CC, cellular component.

Following inoculation, the infection attempt of *Botrytis* instigated arrays of responses from the grapevine inflorescence. As shown in Table 3 and 4, signaling and receptors were differentially expressed within 24 hpi, which are known to involve in immune response to pathogens. Over-expressing *WAK1* enhanced resistance to *B. cinerea* in *Arabidopsis* (Brutus *et al.*, 2010). Conversely, *BAK1* mutant of *Arabidopsis* induced susceptibility to *Sclerotinia sclerotiorum* and *B. cinerea* (Kemmerling *et al.*, 2007). The expression of *BAK1* and *WALK1* membrane receptors increased in *V. pseudoreticulata* in response to *E. necator* (Weng *et al.*, 2014) and in lettuce due to *B. cinerea* (De Cremer *et al.* 2013). The upregulation of these

genes in this study indicated that the plant recognized *Botrytis* intrusion to put defense responses in line. Consequently, quick and strong induction of PR proteins and accumulation of stress related secondary metabolites, as well as cell wall fortification were deployed as major defense responses to halt the infection.

Genes encoding prominent transcription factors (TF), such as WRKY and MYB TFs, known to involve in plant-pathogen interaction, were also differentially expressed. From the qPCR assay, the transcriptional profile of *VvWRKY33* was higher at 12 and 24 hpi (as compared to mock-treated samples)

and dropped to the control level at later time points, 48 hpi and beyond, while the pathogenesis-related (PR) proteins were always higher than control, except *VvPR10.1* at 48 hpi (Figure 5A). A very quick induction of *VvPR10.1* and *VvPR10.3*, coincided with *VvWRKY33*, suggested that these PR proteins involved in grapevine flower resistance against *B. cinerea*. Merz *et al.* (2015) reported that *VvPR10.1* involves in *P. viticola* resistance of grapevine under the regulation of *VvWRKY33*. *WRKY33* in *Arabidopsis*, a functional homology of *VvWRKY33*, confer resistance to *B. cinerea* by regulating redox homeostasis, phytohormonal signaling,

and biosynthesis of phytoalexin (Birkenbihl *et al.*, 2012). The other induced PRs (Table 4), such as chitinases and glucanase, both hydrolytic enzymes, contribute to grapevine resistance by degrading structural components in fungal cell walls (Giannakis *et al.*, 1998). Thaumatin protein has strong anti-fungal activity in vitro by blocking the growth of mycelia of *Phomopsis viticola* and *B. cinerea* (Monteiro *et al.*, 2003), implying to have role in resistance of grapevine against fungal pathogens. The other defense mechanism got activated was the secondary metabolite biosynthesis.

Table 4. Selected *Botrytis*-induced genes in grapevine flower at 24 and 96 hpi (with P-value of  $\leq 0.05$  and absolute fold change of  $\geq 1.5$ )

| Gene Id           | Fold change (log2) |        | Functional annotation                                             | Category                                  |
|-------------------|--------------------|--------|-------------------------------------------------------------------|-------------------------------------------|
|                   | 24 hpi             | 96 hpi |                                                                   |                                           |
| VIT_02s0234g00130 | 1.60               |        | Ethylene responsive element binding factor 1                      | Recognition & signaling                   |
| VIT_11s0016g00710 | 0.83               |        | Jasmonate ZIM-domain protein 1                                    |                                           |
| VIT_00s0258g00140 | 1.26               |        | Brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1) |                                           |
| VIT_17s0000g04400 | 1.38               |        | Wall-associated kinase 1 (WAK1)                                   | Cell wall                                 |
| VIT_14s0128g00970 | 2.75               | 1.40   | Germin-like protein 3                                             |                                           |
| VIT_06s0009g02560 | 3.21               |        | Pectinesterase family                                             |                                           |
| VIT_06s0004g01990 | 4.87               | 3.15   | Proline-rich extensin-like family protein                         | Response to stress & secondary metabolism |
| VIT_11s0149g00280 | 2.13               |        | Chitinase A                                                       |                                           |
| VIT_08s0007g06060 | 1.60               |        | Beta 1-3 glucanase                                                |                                           |
| VIT_05s0094g00320 | 2.65               |        | Chitinase, class IV                                               |                                           |
| VIT_07s0005g02560 | 2.32               |        | Chitinase Class I                                                 |                                           |
| VIT_02s0025g04230 | 2.20               |        | Thaumatin                                                         |                                           |
| VIT_03s0088g00890 | 1.42               |        | Pathogenesis related protein 1 (PR1)                              |                                           |
| VIT_01s0010g02020 | 7.12               | 2.09   | Peroxidase                                                        |                                           |
| VIT_16s0039g01280 | 5.40               |        | Phenylalanin ammonia-lyase                                        |                                           |

The other spectrum of defense mechanism activated following *Botrytis* inoculation was the secondary metabolism. Genes encoding stilbene biosynthesis, *VvSTS29* and *VvSTS41*,

and their regulator *VvMYB14* TF were differentially regulated (Figure 5B). Stilbenes have phytoalexin property (Favaron *et al.*, 2009). Fortification of cell wall was also

hinted from the upregulation of genes involved in monolignol biosynthesis pathway, a precursor for lignin synthesis (Figure 5C). Genes encoding 4-coumarate-CoA ligase (Vv4CL), an enzyme in the upstream of the pathway, cinnamoyl CoA reductase (VvCCR), the first enzyme specific to monolignol synthesis, and Cinnamyl alcohol dehydrogenase (VvCAD), the final enzyme in the sequential reactions in the biosynthesis, were all differentially regulated at 12 and 24 hpi, VvCAD at 48 hpi as well (Figure 5C). Taken together, the grapevine fruitlets undertook a remarkable transcriptional reprogramming towards defense at the earlier time of *Botrytis* infection, i.e. within 24 hpi.

Genes encoding for the TF regulating stilbene biosynthesis, VvMYB14 (Höll *et al.*, 2013), and VvSTS29 and VvSTS41 were switched on following the infection. Studies have shown that stilbenes have phytoalexin nature and known to affect the growth of *B. cinerea* (Favaron *et al.*, 2009). A transgenic *Arabidopsis*, which overexpresses a STS gene, increased its resistance to *Colletotrichum higginsianum* (Liu *et al.*, 2011). In line with this, over-expressing the STS gene helped transgenic grapevine plants to resist *B. cinerea* colonization (Dabauza *et al.*, 2014).

Reinforcing cell wall denies pathogens access to epidermal layer. The upregulation of monolignol genes were shown to help resistance in wheat against *Blumeria graminis* f. sp. *Tritici*

(Bhuiyan *et al.*, 2009). Whereas, silencing certain key genes of the monolignol biosynthesis pathway, such as PAL and CAD, compromised penetration resistance of wheat to the pathogen (Bhuiyan *et al.*, 2009). In the grapevine fruitlets, the analyzed monolignol biosynthesis genes (Figure 5C), involved from upstream to downstream of the pathway, were differentially regulated following *B. cinerea* inoculation starting from as early as 12 hpi, suggesting cell wall apposition was in effect at such early time of interaction.

In conclusion, the microscopic and transcriptomic studies of this research provided good insight about the interaction between *B. cinerea* and grapevine inflorescence during the first 96 hpi. Within 24 hpi, *B. cinerea* genes required for establishing successful infection, such as those encoding cell wall degrading enzymes and phytotoxins, were induced. Wall associated receptor kinases of the inflorescences recognized the infection attempt and activated signal transduction, to reprogram inflorescences' transcriptome to "defense-oriented mode". As a result, enhanced expression of PR superfamily, stilbenoids (phytoalexins), and lignin biosynthesis (to stiffen cell wall) were put in place to tackle *B. cinerea* advancement. These defense responses were effective as at 96 hpi the numbers of *in planta* detected *B. cinerea* genes were evidently low, as compared to the initial infection stage; suggesting the

fungus switched to basal biological activity, i.e. entered to quiescent state. This was further confirmed by reduced number of differentially regulated defense related genes of the inflorescences at 96 hpi. The expression profiles of the defense responses of grapevine inflorescence that put *B. cinerea* into quiescent

should be further studied to better understand their involvement in relation to ontogenic resistance. Molecular crosstalk of *B. cinerea* and grape berry at different growth stages and infection states should be further investigated for devising infection state/growth stage specific management of the fungus.

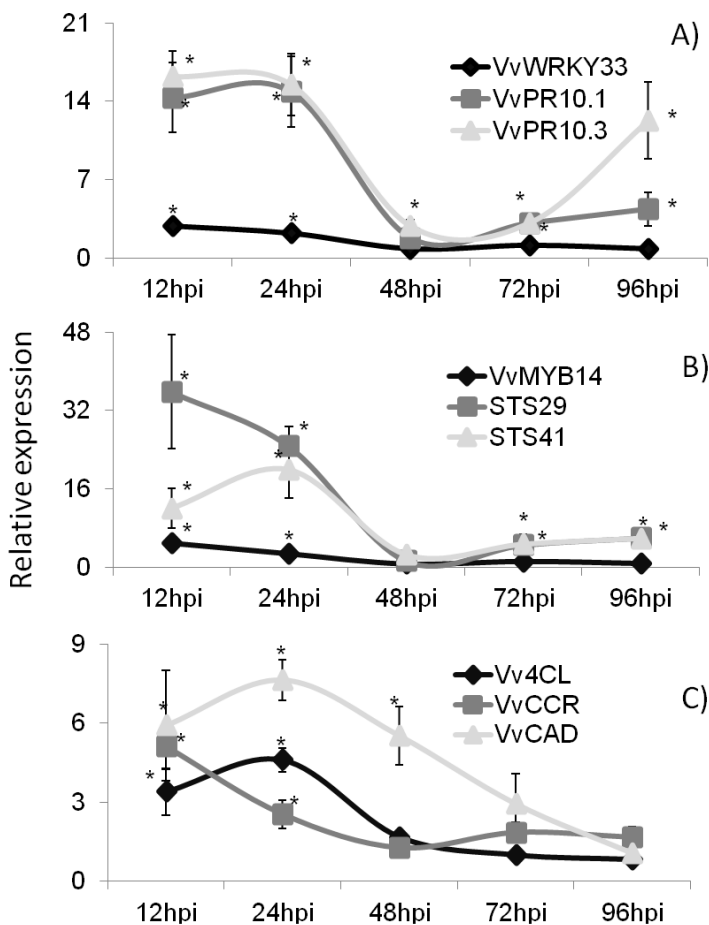


**Figure 5.** The expression profile of transcription factors and defense related genes [at 12, 24, 48, 72, and 96 hours post inoculation (hpi)]. **(A)** Expression profile of VvWRKY33 TF and coexpressed PR genes, VvPR10.1, and VvPR10.3. **(B)** Expression of Stilbene synthase genes VvSTS29 and VvSTS41, and their regulator Myb14 transcription factor. **(C)** Expression of critical enzymes in monolignol biosynthetic pathway, VvCCR (cinnamoyl CoA reductase), Vv4CL (4-coumarate-CoA ligase), and VvCAD (cinnamyl alcohol dehydrogenase). Error bar is the standard error of the mean of three biological replicates. Asterisks (\*) indicate statistically significant difference ( $P < 0.05$ ) between mock- and *Botrytis cinerea*- inoculated samples within post-inoculation hours using unpaired heteroscedastic Student's t test.

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# Nature of Gene Action for Yield and Yield Related Traits in Sesame (*Sesamum indicum* L.) Genotypes

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## Abstract

An understanding of the nature of gene action is a prerequisite for any successful breeding program. The aim of this study was to determine the genetic system governing yield and yield related traits in sesame (*Sesamum indicum* L.) genotypes. Ten sesame lines viz., EW002, BG006, EW023-2, EW006, EW003-1, EW019, Obsa, Dicho, Wama and EW010-1 were crossed in year 2011 in a complete diallel fashion. Seeds of all  $F_1$  and their parents were planted at Wama research sub sites in Randomized Complete Block Design with three replications. Data for days to flowering, branches per plant, plant height, capsules per plant and seed yield per plant were recorded. The result of this study showed that additive and dominant gene effects were important for all the traits studied. Both maternal (c) and reciprocal (d) effects were significant for days to flowering and grain yields per plant. Medium narrow sense heritability was observed for plant height and branches per plant. In this study, the predominance of over dominance gene action was involved in all traits. This showed that there is high scope of heterosis breeding. On the other hand, recurrent selection is likely lead to overall population improvement of this crop for grain yield and yield related traits.

**Keywords:** Additive, Dominance, Gene effects, Genetic analysis, Inheritance

## Introduction

The choice of an efficient breeding procedure depends on the knowledge of the genetic system controlling the trait to be selected (Eshghi and Akhundova, 2009). An understanding of the nature of gene action is a prerequisite for any successful breeding program (Anyanga *et al.*, 2016). The main effort of sesame breeder is the detection of favorable

genes and to assemble them into a particular genotype using the most suitable combination. So it is necessary to introduce different genes which are known to be yield contributor. The study of inheritance pattern and analysis of gene action is very important to make decision about selection of suitable parents.

The diallel analysis techniques developed by Hayman (1954 a, b) and

Jinks (1954, 1956) provide a reliable mechanism to properly understand the genetic system and gene action involved in the expression of important plant attributes. The diallel cross procedures provides statistical approach of genetic study which is widely used by breeders for analyzing metric traits in different species. It furnishes logical information about the genetic architecture of the plant and heredity behavior of the parameters under study in early generation like  $F_1$  and  $F_2$ . It also describes how to measure additive and dominance variation and the relative dominance properties of the parental lines.

The study on the genetic analysis of sesame would help the breeder to formulate appropriate selection procedure for screening of these inbred lines and the evaluation of their progenies for various purposes (Swain *et al.*, 2001). It provides heritability estimates of the character, which helps to predict progress through selection. The character showing high heritability could be improved through direct selection. On the other hand, in the case of progenies involving a greater non-additive genetic variance including dominance, over dominance, epistasis and linkage; selection for desirable genotypes is not straightforward. There are number of biometrical approaches available to estimate genetic component of variations (Mather and Jinks, 1971) but there have been very few attempts to apply them to the phenomenon of genetic analysis of sesame. In

Ethiopia, information on the inheritance of yield and yield related traits in sesame is lacking. Therefore, this experiment was designed to assess the nature and magnitude of gene actions controlling the inheritance of seed yield and yield related traits in sesame genotypes.

## Materials and Methods

The experimental material comprised of ten morphologically and genetically diverse sesame genotypes viz., EW002, BG006, EW023-2, EW006, EW003-1, EW019, Obsa, Dicho, Wama and EW010-1. Varieties such as Obsa and Dicho are released cultivars while genotypes such as EW002, BG006, EW023-2, EW006, EW003-1, EW019 and Wama are elite breeding lines. These genotypes were primarily collected from Western part of Ethiopia. A single plant from each genotype was selected and multiplied in 2010 off-season to get pure lines. These ten genotypes were crossed in 10 x 10 diallel mating design including reciprocal crosses in 2011 main cropping season. Seeds of all the  $F_1$ s and their parents were planted on 14<sup>th</sup> June 2012 at Wama testing sites of the Bako Agricultural Research Center (BARC) in a Randomized Complete Block Design (RCBD) with 3 replications. Wama has an altitude of 1436 meter above sea level (masl). Each plot consisted of a single row of 5 m length with 50 cm and 25 cm inter and intra row spacing, respectively. The seeds were drilled in each row at

seeding rate of  $5 \text{ kg ha}^{-1}$ . Twenty days after planting, the plants were thinned out to adjust for required population per hectare. Nitrogen fertilizer in the form of Urea was applied as side dressing four weeks after emergence at the rate of  $50 \text{ kg ha}^{-1}$ . Hand weeding was carried out four times at two weeks interval starting twenty days after planting. Data for days to flowering was recorded on a plot basis while the remaining data were recorded on plant basis. Observations were made on ten randomly selected plants for branches per plant, plant height, capsules per plant, and seed yield per plant.

Analysis of variance (ANOVA) was performed as per the suggestions of Hayman (1954 a, b) and Jinks (1954,1956). In the first phase, formal analysis of variance of the diallel table divided the family mean effects into additive (a) and dominance (b) components. It also detects maternal (c) and reciprocal (d) effects. The dominance (b) component was further separated into directional dominance effects (b1, the mean deviation of the crosses from the mid parent values); effect due to unequal contribution of the dominance alleles by parents (b2) and the specific gene interaction (b3) which is termed as specific combining ability (Griffing, 1956a) and refers to those cases in which certain parental combinations perform relatively superior or inferior than expectation based on the average potential of the genotype involved. Each main effect was tested against its own interaction

over blocks. If 'c' maternal difference was significant 'a' (additive effects) was retested against 'c'. If 'd' reciprocal effects was significant 'b' (dominant effects) and its components were retested against 'd'.

The second step of analysis was the computation of the variance of each array ( $V_r$ ), the covariance of all the offspring included in each parental array with the non-recurrent parents ( $W_r$ ) and the variance of the parental means ( $V_0L_0$ ). In addition, the means of the array variance ( $V_1L_1$ ), the variance of the mean of array ( $V_0L_1$ ) and the mean of the array covariance ( $W_0L_0$ ) were also computed. The validity of the assumption regarding adequacy of additive dominance model was tested before undertaking any numerical analysis.

The genetic parameters such as E (environmental variance from analysis of variance), D (estimate of additive and some portions of additive x additive genetic variance), F (estimate of relative frequency of dominant and recessive alleles in the parent),  $H_1$  and  $H_2$  (estimate of dominance and dominance x dominance interactions, respectively),  $h^2$  (dominant effect as algebraic sum over all loci in heterozygous phase in all crosses provides the direction of dominance i.e. positive sign shows that direction of dominance being towards parents and the negative sign shows towards off springs),  $H^2/4H_1$  and  $[(4DH_1)^{1/2} + F]/[(4DH_1)^{1/2} - F]$  (KD/KR) provides the proportion of dominant

and recessive genes in the parents,  $(H_1/D)^{1/2}$ , (as mean degree of dominance), and heritability in narrow sense were computed according to Mather and Jinks (1982). The correlation between parental order of dominance ( $W_r+V_r$ ) and parental measurement ( $Y_r$ ) ( $r(Y_r, W_r + V_r$  (the prediction for measurement of completely dominant and recessive parents) were also estimated as suggested by Mather and Jinks (1982). The minimum number of gene which exhibited some degrees of dominance and effective factor ( $k$ ) were also computed as suggested by Hayman (1954a), Jinks (1956) and Mather's (1949).

## Results and Discussion

For all traits considered in the preset study, mean squares were highly significant, indicating that there is a considerable genetic variability among the parents and the crosses. In addition, these genotypes seem to have different genes controlling the investigated traits (Table 1). These results are also confirmed by earlier reports (El-Bramawy, 2010; Pham *et al.*, 2010). The  $W_r-V_r$  mean square was non-significant when tested against error mean square for five traits, indicating the validity of additive dominance model (Table 2).

Table 1. Mean squares values for five traits considered in the current study

| Source of Variation | df  | Mean squares |          |         |            |          |
|---------------------|-----|--------------|----------|---------|------------|----------|
|                     |     | DF           | PH       | BP      | CP         | YP       |
| Rep                 | 2   | 130.04**     | 668.84** | 71.61** | 25632.67** | 261.34** |
| Genotype            | 99  | 9.84**       | 324.18** | 5.23**  | 3299.79**  | 42.231** |
| Error               | 198 | 4.2          | 199.30   | 2.08    | 1299.26    | 15.27    |

\*\* Significant at  $p < 0.01$  level; df=degree of freedom, DF=days to 50% flowering; PH=plant height; BP=branches per plant; CP=capsules per plant and YP=yield per plant

Table 2. Results of analysis of variance for ( $W_r-V_r$ ) in sesame genotypes

| Source    | df | Mean squares |           |        |              |         |
|-----------|----|--------------|-----------|--------|--------------|---------|
|           |    | DF           | PH        | BP     | CP           | YP      |
| Rep       | 2  | 2.05         | 9779.42   | 5.87   | 36715554.9   | 93.3    |
| $W_r-V_r$ | 9  | 2.29ns       | 5657.11ns | 1.39ns | 37003221.8ns | 109.4ns |
| Error     | 18 | 3.00         | 4673.82   | 1.51   | 39604250.0   | 64.9    |

\*\* Significant at  $p < 0.01$  level; DF=days to 50% flowering; PH=plant height; BP=branches per plant; CP=capsules per plant and YP=yield per plant

Hayman's ANOVA for studied traits were presented in Table 3. Item 'a' was significant for all traits except for days to 50% flowering, demonstrating that the additive genetic variance was important for these traits.

Praveenkumar *et al.* (2012) were also reported additive gene effect for plant height, and seed yield per plant in sesame. Contrary to the present result, Sakhiya (2013) reported significant additive gene effect in sesame for days

to 50 % flowering. Mather & Jinks (1982) observed maternal effects due to the significance of item 'c', which suggested that re-testing of 'a' against mean square of 'c'. After re-testing of 'a' against that of 'c', the significance level of 'a' item remained unchanged for branches per plant and yield per plant, demonstrating that maternal effects has not masked the effects of genes which were acting additively. Item 'b<sub>1</sub>' was non-significant for all traits, indicating that dominance was ambi-directional for these traits. Among parents, asymmetrical gene distribution was evident due to significant of 'b<sub>2</sub>' for days to flowering, plant height and branches per plant. This unequal distribution of dominant and recessive alleles genes clearly suggests that some parents have considerably more dominant alleles than others for these traits. On the other hand, the parents contained uniform distribution of dominant genes for capsule per plant and yield per plant as item 'b<sub>2</sub>' was non-significant for these traits. For all traits, significance of 'b<sub>3</sub>' items suggested that the genes with specific dominant gene effects were present. Item 'b' was highly significance for all traits, indicating the presence of dominant genes effects. Aladi Abatchoua *et al.* (2014)) also reported dominant gene action for these traits. For days to flowering, the reciprocal effects had masked the dominant genes as implied by the reduction to non-significant after retesting of b<sub>2</sub>, b<sub>3</sub>

and b items against item 'd'. Maternal effects were exhibited due to significance of items 'c' for days to flowering, branches per plant and yield per plant. The presence of reciprocal effect was revealed by significance of 'd' for days to flowering, capsules per plant and yield per plant. The choice of female parent is critical in a breeding program (Arunga *et al.*, 2010). In the present study, both additive and dominant genes were involved in controlling the traits. Anyanga *et al.* (2016) also observed that both additive and non-additive gene actions were important for these traits.

Table 4 shows the estimates of genetic parameters for yield and yield related trait. The additive variance (D) was significant for plant height, branches per plant and yield per plant showing that successful selection could be practiced for these traits. The dominance variance component (H<sub>1</sub>) was significant for all traits except for days to flowering, suggesting that, the non-additive type of gene actions were involved in the inheritance of these characters. Days to flowering, branches per plant, capsules per plant and yield per plant are under the influence of dominant gene effects as shown by the significant value of non-additive genetic effects (H<sub>2</sub>). The higher value of H<sub>1</sub> than D revealed that, non-additive effects were more pronounced as compared to additive gene effects.

Table 3. Hayman's ANOVA table in 10x10 diallel cross of sesame genotypes considered in this study

| Item               | Df  | Days to 50% flowering |          |                    | Plant height |          | Branches per plant |          |                    |
|--------------------|-----|-----------------------|----------|--------------------|--------------|----------|--------------------|----------|--------------------|
|                    |     | MS                    | F- ratio | Retested against d | MS           | F- ratio | MS                 | F- ratio | Retested against c |
| a                  | 9   | 11.12                 | 1.90ns   |                    | 812.20       | 3.84**   | 16.48              | 16.55**  | 3.82*              |
| b <sub>1</sub>     | 1   | 9.60                  | 2.09ns   |                    | 98.61        | 1.40ns   | 7.89               | 13.94ns  |                    |
| b <sub>2</sub>     | 9   | 13.19                 | 3.06*    | 1.37ns             | 728.35       | 4.79**   | 9.02               | 4.85**   |                    |
| b <sub>3</sub>     | 35  | 7.96                  | 2.07**   | 0.83ns             | 282.50       | 1.86*    | 4.48               | 1.65**   |                    |
| b                  | 45  | 9.04                  | 2.29**   | 0.94ns             | 367.59       | 2.49**   | 5.46               | 2.19**   |                    |
| c                  | 9   | 13.50                 | 4.01**   |                    | 232.00       | 0.61ns   | 4.32               | 3.02*    |                    |
| d                  | 36  | 9.60                  | 2.22**   |                    | 170.95       | 0.78ns   | 2.37               | 1.18ns   |                    |
| Total              | 99  | 9.84                  |          |                    | 324.18       |          | 5.23               |          |                    |
| a x B              | 18  | 5.85                  |          |                    | 211.61       |          | 1.0                |          |                    |
| b <sub>1</sub> x B | 2   | 4.58                  |          |                    | 70.58        |          | 0.66               |          |                    |
| b <sub>2</sub> x B | 18  | 4.28                  |          |                    | 152.72       |          | 1.86               |          |                    |
| b <sub>3</sub> x B | 70  | 3.85                  |          |                    | 151.69       |          | 2.72               |          |                    |
| b x B              | 90  | 3.96                  |          |                    | 150.09       |          | 2.50               |          |                    |
| c x B              | 18  | 3.37                  |          |                    | 356.64       |          | 1.43               |          |                    |
| d x B              | 72  | 4.32                  |          |                    | 218.39       |          | 2.00               |          |                    |
| B x interaction    | 198 | 4.2                   |          |                    |              |          | 2.08               |          |                    |

\*, \*\* = significant at P < 0.05 and P < 0.01, respectively, B= block, df: Degree of freedom a = additive effects of genes; b = dominant effects of genes; b<sub>1</sub> = mean dominance effects; b<sub>2</sub> = additional dominance deviation due to the parents, b<sub>3</sub> = residual dominance effects; c=maternal effect; d=reciprocal difference

| Item               | Df  | Capsules per plant |          |                    | Yield per plant |         |                  |                  |
|--------------------|-----|--------------------|----------|--------------------|-----------------|---------|------------------|------------------|
|                    |     | MS                 | F- ratio | Retested against d | MS              | F-ratio | Tested against c | Tested against d |
| a                  | 9   | 4398.84            | 0.231ns  |                    | 48.3            | 3.11**  | 16.1*            |                  |
| b <sub>1</sub>     | 1   | 2066.31            | 1.09ns   |                    | 18.6            | 0.85ns  |                  |                  |
| b <sub>2</sub>     | 9   | 2870.53            | 2.06ns   |                    | 37.2            | 2.29ns  |                  |                  |
| b <sub>3</sub>     | 35  | 3889.94            | 3.04**   | 3.59**             | 51.0            | 3.20**  |                  | 2.9479**         |
| b                  | 45  | 3645.54            | 2.77**   | 3.36**             | 47.5            | 2.95**  |                  | 2.7456**         |
| c                  | 9   | 2490.54            | 1.36ns   |                    | 37.0            | 12.33** |                  |                  |
| d                  | 36  | 2795.15            | 2.58**   |                    | 35.4            | 2.04**  |                  |                  |
| Total              | 99  | 3299.79            |          |                    | 42.23           |         |                  |                  |
| a x B              | 18  | 18794.38           |          |                    | 15.5            |         |                  |                  |
| b <sub>1</sub> x B | 2   | 1880.64            |          |                    | 21.8            |         |                  |                  |
| b <sub>2</sub> x B | 18  | 1390.00            |          |                    | 16.2            |         |                  |                  |
| b <sub>3</sub> x B | 70  | 1277.74            |          |                    | 15.9            |         |                  |                  |
| b x B              | 90  | 1313.60            |          |                    | 16.1            |         |                  |                  |
| c x B              | 18  | 1818.63            |          |                    | 3.0             |         |                  |                  |
| d x B              | 72  | 1083.27            |          |                    | 17.3            |         |                  |                  |
| B x interaction    | 198 | 610.85             |          |                    | 159.16          |         |                  |                  |

\*, \*\* = significant at P < 0.05 and P < 0.01, respectively, B= block, df: Degree of freedom a = additive effects of genes; b = dominant effects of genes; b<sub>1</sub> = mean dominance effects; b<sub>2</sub> = additional dominance deviation due to the parents, b<sub>3</sub> = residual dominance effects; c=maternal effect; d=reciprocal difference

The dominant effect ( $h^2$ ) was significant and positive for yield per plant. The results indicated that the mean direction of dominance was positive for this trait. Sakhiya (2013)

has also reported similar results in sesame. For days to flowering, branches per plant and capsules per plant, dominant gene effect was positive but not significant. The values

of F were found to be non-significant for capsule per plant and yield per plant, which indicated an equal distribution of dominant and recessive genes in the parents. Similar results have been also reported by Mothilal and Manoharan (2005) for seed yield per plant, and by Sakhiya (2013) for number of capsules per plant. The value of 'F' was negative for days to 50% flowering, suggesting that this trait was under influence of recessive genes. The involvement of the environmental effects in the expression of all traits was revealed by the highly significant of environmental variance (E) for all traits except days to flowering. However, environmental variance was less than additive and dominant variances. The proportional value of  $(H_1/D)^{1/2}$  indicated the control of over dominance gene action for all studied traits. This value suggested that the dominance component is relatively more important, so delayed selection in segregating generations should be preferred for the traits. Furthermore, this result showed plenty of possibilities for heterosis breeding in sesame. Significant over dominance for plant height, days to 50% flowering, number of capsules per plant, number of seeds per capsule and seed yield per plant were earlier reported by Praveenkumar *et al.* (2012). Biparental mating or recurrent selection in segregating materials followed by conventional selection scheme is likely to lead to improvement in the characters of the studied sesame genotypes. The proportions of dominant genes with

positive or negative effects in parents ( $H_2/4H_1$ ) were different from 0.25 for all traits; hence, dominant genes having increasing and decreasing effects in these traits are irregularly distributed in parents.

The inferences drawn from the F value was also confirmed by the proportion of dominance to recessive alleles in the parents as measured by the value of KD/KR, which was more than unit for plant height, branches per plant, and capsules per plant and yield per plant. Symmetry of dominant and recessive allele's distribution in parents is further established by relative sizes of  $H_1$  and  $H_2$ . The value of  $H_1$  and  $H_2$  were not equal for all traits, indicating the asymmetric distribution of dominant and recessive genes. The value of K ( $h^2/H_2$ ) was greater than one for yield per plant, suggesting that more than one group of genes exhibiting dominance governed that character. For the other traits, value of K was less than one. Thus, the value of K did not provide any valid interpretation for all the traits about the group of genes exhibiting dominance. The ratio could be under estimated when the dominance effects of all the genes concerned are not equal in size and distribution, when the distribution of genes is correlated (Jinks, 1954), or when the complementary gene interactions occur (Mather and Jinks, 1971). Parents possess mostly negative genes in dominant form for branches per plant, capsule per plant and yield per plant as indicated by the positive

correlation between parental value ( $V_r$ ) and recessive factor ( $W_r + V_r$ ). These results are in harmony with the findings of El-Bramawy (2010). The association of these yield components with recessive genes might present some difficulties for selection during the early generations. On the other hand, the increasing genes were dominant for days to flowering and yield per plant as revealed by negative correlation between parental value ( $V_r$ ) and recessive factor ( $W_r + V_r$ ).

Narrow sense heritability ranging from 6.3 to 25% was observed for all traits, which suggested the need for

population improvement or a good scope for the development of hybrids. Low narrow sense heritability was detected for seed yield per plant and days to flowering. Medium heritability was noticed for plant height and branches per plant. On the contrary, Vekaria *et al.* (2015) had reported high narrow sense heritability for plant height and branches per plant in sesame. In the present study, since non-additive genetic effects played major role along with low to medium heritability for the traits, little response to selection is expected in the early generations.

Table 4. Estimates of genetic parameters for yield and yield related traits in sesame genotypes

| Genetic parameters                    | DF                           | PH                   | BP                 | CP                     | YP                 |
|---------------------------------------|------------------------------|----------------------|--------------------|------------------------|--------------------|
| D                                     | 2.207 $\pm$ 3.96             | 64.95 $\pm$ 31.13**  | 1.44 $\pm$ 0.36**  | 562.6 $\pm$ 520.12     | 7.86 $\pm$ 3.9**   |
| H <sub>1</sub>                        | 10.68 $\pm$ 8.43             | 239 $\pm$ 66.25**    | 3.453 $\pm$ 0.77** | 2417 $\pm$ 1107**      | 25.054 $\pm$ 8.3** |
| H <sub>2</sub>                        | 14.57 $\pm$ 7.16**           | 107.84 $\pm$ 56.31   | 1.792 $\pm$ 0.66** | 2794.92 $\pm$ 940.94** | 19.87 $\pm$ 7.02** |
| h <sup>2</sup>                        | 1.5 $\pm$ 4.79 <sub>ns</sub> | -12.60 $\pm$ 37.68   | 0.61 $\pm$ 0.44    | 126.08 $\pm$ 629.64    | 33.87 $\pm$ 4.7**  |
| F                                     | -2.42 $\pm$ 9.13             | 149.98 $\pm$ 71.82** | 2.194 $\pm$ 0.84** | 0.36 $\pm$ 12.00       | 11.008 $\pm$ 9.0   |
| E                                     | 1.82 $\pm$ 1.19              | 58 $\pm$ 9.38**      | 0.926 $\pm$ 0.11** | 514.19 $\pm$ 156.82**  | 5.91 $\pm$ 1.2**   |
| Proportional Values                   |                              |                      |                    |                        |                    |
| (H <sub>1</sub> /D)1/2                | 2.19                         | 1.92                 | 2.232              | 2.072                  | 1.785              |
| H <sub>2</sub> /4H <sub>1</sub>       | 0.341                        | 0.112                | 0.129              | 0.289                  | 0.198              |
| KD/KR                                 | 0.63                         | 4.008                | 2.931              | 1.003                  | 2.290              |
| K (h <sup>2</sup> /H <sub>2</sub> )   | 0.1029                       | -0.116               | 0.340              | 0.45                   | 1.704              |
| r(Yr,W <sub>r</sub> +V <sub>r</sub> ) | 0.16                         | -0.74*               | 0.20               | 0.20                   | -0.33              |
| Heredity coefficients:                |                              |                      |                    |                        |                    |
| h <sup>2</sup> <sub>ns</sub> (%)      | 6.3                          | 21.4                 | 25.0               | 7.1                    | 9.0                |

ns, \*\*, non-significant and significant at 0.01 levels, respectively; DF=days to 50% flowering ,PH= plant height , BP=branches per plant, CP=capsules per plant, YP= yield per plant ;D=estimate of additive variance H<sub>1</sub> and H<sub>2</sub>= (estimate of dominance and dominance x dominance interactions, respectively), h<sup>2</sup> =dominant effect as algebraic sum over all loci in heterozygous phase in all crosses provides the direction of dominance i.e. positive sign shows that direction of dominance being towards parents and the negative sign shows towards offsprings),E= environmental variance from ANOVA), F= estimate of relative frequency of dominant and recessive alleles in the parent).

## Conclusion

For all traits, the result of the current study showed that both additive and dominant gene effects were important in sesame genotypes considered. Days to flowering, branches per plant and yield per plant were significantly controlled by maternal and reciprocal effects. Reciprocal effects were important for traits viz., days to flowering, capsules per plant, and yield per plant. For plant height and branches per plant, medium narrow sense heritability was observed. Over dominance was predominant in all traits and hence recurrent selection

scheme is likely to lead to improvement of the crop for yield and yield related traits. Generally, the present study indicated that, there is high scope of heterosis breeding for grain yield in sesame genotypes.

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# Growth Gas Exchange Parameters of Maize with Elevated Atmospheric Carbon Dioxide and Temperature

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## Abstract

Temperature and carbon dioxide (CO<sub>2</sub>) are two important parameters related to climate change, which affect gaseous exchange and yield parameters of many cereal food crops. In this study, an experiment was conducted growing maize (*Zea mays* L.) in open top chambers (OTCs) to determine the effects of elevated CO<sub>2</sub> and temperature on gaseous exchange parameters (leaf photosynthetic rate (P<sub>N</sub>), stomatal conductance (gs), intercellular CO<sub>2</sub> concentration (Ci), transpiration rate (Tr) and water use efficiency (WUE) of maize crop in Indian Agricultural Research Institute (IARI), New Delhi, India. Maize (var. PEHM 5) was grown with two levels of CO<sub>2</sub> ambient (400 ppm) and elevated (550 ± 20 ppm) and three levels of temperature i.e., ambient, ambient +1.5 °C and ambient +3.0 °C during {July–October} seasons of 2013 and 2014. The two years average data indicated that the interactive effects of elevated CO<sub>2</sub> with 1.5 and 3.0 °C temperature rise increased photosynthetic rate by 24.32 & 37.5%, stomatal conductance by 50.0% & 87.5%, intercellular CO<sub>2</sub> concentration by 55.0% & 77.16%, transpiration rate by 44.0% & 102.16%, but decreased intrinsic water use efficiency by 7.46% & 28.7%, respectively. On the contrary, elevated CO<sub>2</sub> with 1.5 and 3.0 °C temperature rise decreased photosynthetic rate by 4.67 & 8.34%, stomatal conductance by 34.78% & 43.48%, transpiration rate by 25.17% & 20.52%, but increased intrinsic water use efficiency by 28.6% & 17.23%, respectively. The interactive effects of elevated CO<sub>2</sub> with 1.5 °C temperature rise resulted in 10.81% decrease and elevated CO<sub>2</sub> with 3.0 °C temperature rise resulted in a 14.47% increase in intercellular CO<sub>2</sub> concentration as compared to ambient CO<sub>2</sub> and temperature. Elevated temperature by 1.5°C and 3.0 °C at elevated CO<sub>2</sub> had no effect on photosynthetic rate and intercellular CO<sub>2</sub> concentration. In addition, elevated temperature by 3.0 °C at elevated CO<sub>2</sub> had no effect on intrinsic water use efficiency as compared to ambient CO<sub>2</sub> and temperature treatment. From this study it can be concluded that elevated levels of both temperature and CO<sub>2</sub> have significant effects on maize growth gas exchange parameters.

**Keywords:** Elevated Carbon Dioxide, Elevated temperature, Maize, Photosynthetic Rate, Water Use Efficiency

## Introduction

Maize is a  $C_4$  crop and is one of the most versatile cereal crops, cultivated in nearly 150 million hectares in more than 160 countries, contributing 36% (782 million ton (Mt) of the global grain production (FAOSTAT, 2010). Almost 70% of total maize production is in the developing world. In India, maize is grown throughout the year but predominantly as a kharif (June-October) crop with 85% of the area under cultivation in the season. Maize is the third most important cereal crop after rice and wheat in the country accounting for about 9% of total food grain production with a production of 21.28 Mt and productivity of  $2.5 \text{ t ha}^{-1}$  (CMIE, 2010; Prathyusha *et al.*, 2013; Venkata *et al.*, 2014).

The global climate is changing at an alarming rate due to increased emission of greenhouse gases (GHGs) such as carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ). The atmospheric concentration of  $\text{CO}_2$  was around 280 ppm in 1750 AD (pre-industrial era). Currently, it is more than 400 ppm i.e., increased by 40% with a consequent warming of the atmosphere by  $0.84^\circ\text{C}$  (IPCC, 2014). It is projected that with the rise of GHGs, global average temperature will rise by  $3.7$  to  $4.8^\circ\text{C}$  by the end of 21<sup>st</sup> century (IPCC, 2014). Crop production will be affected by global warming which is caused by two major factors i.e., elevated atmospheric  $\text{CO}_2$  and temperature. Increased concentration of  $\text{CO}_2$  will

promote growth of many plants through enhanced photosynthesis. This positive effect of  $\text{CO}_2$  on plant growth is more pronounced in  $C_3$  crops such as wheat but less notable in  $C_4$  crops like maize (Ghannoum *et al.*, 2000). However, elevated temperature will cause heat injury and physiological disorders resulting in reduced yield (Johkan *et al.*, 2011). The increased temperature as a result of elevated  $\text{CO}_2$  will have a major influence on regional as well as global crop growth and productivity depending on the locations. Increase in ambient temperature by  $1.0$ - $2.0^\circ\text{C}$  in tropical and subtropical countries like India food grain production is projected to decrease up to 30% (IPCC, 2007; Johkan *et al.*, 2011). On the contrary, the world population is expected to reach nine to ten billion people by 2050 and production of food should increase by 70% to ensure food security, which should be achieved through increase in productivity (FAO, 2017).

Growth gas exchange parameters and productivity of maize is likely be affected by both elevated  $\text{CO}_2$  concentration and temperature. Elevated temperature is expected to influence growth, development, yield and quality of maize negatively (Morison, 1996; IPCC, 2007; Mendelsohn and Dinar, 2009; Pathak *et al.*, 2012). The two most familiar responses to elevated  $\text{CO}_2$  at the leaf level are an increase in the rate of net photosynthesis and a decrease in stomata conductance that leads to

increased crop productivity and water use efficiency. The increase in net photosynthetic rate is much larger in  $C_3$  species (50 to 100%) than in  $C_4$  species (10%) when  $CO_2$  concentration doubles but a substantial decrease in stomatal conductance (30 to 40%) is observed in both types of species including maize crop (Ainworth and Long, 2005). The increasing atmospheric  $CO_2$  concentration have direct and indirect effects on crop plants and thus there are inconsistent reports varying from little positive effect (Ghannoum *et al.*, 2000; Leakley *et al.*, 2004), no effect (Kim *et al.*, 2007) to increase by 50% in growth and yield of maize (Rogers *et al.*, 1983; Samarakoon and Gifford, 1996; Prins *et al.* 2007; Leaky *et al.*, 2004). However, the long-term response remains uncertain due to increase in incidence of extreme weather events such as drought, heat waves, and heavy precipitation, making crop production more unpredictable and difficult (IPCC, 2014). In addition to this, most of the experiments on the impacts of elevated  $CO_2$  and temperature on maize growth and yield parameters used controlled environment facilities such as phytotron and plant growth chambers or crop growth simulation models. There are very limited studies on the impacts of elevated  $CO_2$  concentration and temperature interactions on this

important cereal crop under field conditions (Mendelsohn and Dinar, 2009; Fang *et al.*, 2010, Pathak *et al.*, 2012). Hence, the objectives of the study was to evaluate the impacts of elevated atmospheric  $CO_2$  and temperature on growth gas exchange parameters of maize in north-west India under near natural field conditions.

## Materials and Methods

### Site description

An experiment was conducted at the research farm of Indian Agricultural Research Institute (IARI), New Delhi using open top chambers (OTCs). The site is located at  $28^{\circ} 37' N$  latitude,  $77^{\circ} 12' E$  longitude at an altitude of 228.6 m above mean sea level. The climate of the area is sub-tropical, semi-arid with an average annual rainfall of 750 mm, 80% of which is received during July to October (Table 1). Maximum and minimum temperatures of the site ranged from  $34-35^{\circ}C$  and  $24-26^{\circ}C$ , respectively. The texture of the soil of the experimental site was sandy clay loam with pH 8.43, EC  $0.16 dS m^{-1}$ , organic C 0.45%, available N  $185.1 kg ha^{-1}$ , available P  $25.1 kg ha^{-1}$ , available K  $247.9 kg ha^{-1}$  and dehydrogenase activity  $150 \mu TPF g^{-1} 24 hrs^{-1}$ .

Table 1. Weather during the maize growing periods in 2013 and 2014.

| Month (Year)    | Rainfall (mm) | Temperature (°C) |         | Sun shine (hour) | Relative humidity (%) |
|-----------------|---------------|------------------|---------|------------------|-----------------------|
|                 |               | Maximum          | Minimum |                  |                       |
| July, 2013      | 460.0         | 34.8             | 25.7    | 113.5            | 81.4                  |
| August, 2013    | 521.9         | 32.8             | 25.1    | 101.3            | 88.5                  |
| September, 2013 | 105.9         | 34.5             | 24      | 204.7            | 74.7                  |
| July, 2014      | 228.8         | 35.9             | 26.3    | 136.3            | 75.3                  |
| August, 2014    | 98.9          | 35.8             | 25.8    | 152              | 72.3                  |
| September, 2014 | 123.3         | 34.1             | 24      | 208.3            | 70.6                  |

## Treatments and experimental design

Maize crop (variety PEHM 5) was grown under two levels of atmospheric CO<sub>2</sub> (ambient and 550 ± 20 ppm) and three levels of temperature (ambient, ambient + 1.5 °C and ambient + 3.0 °C) during kharif 2013 and 2014 in OTCs and ambient field condition (Table 2). The experiment was conducted in a two factor completely randomized design (CRD) design with three replications. The maize crop was grown with spacing of 20 cm between plants and 60 cm between rows. Sowing was done on July 9 for the first year (2013) and July 8 for the second year (2014). The crop was grown under two levels of atmospheric carbon dioxide (ambient and 550ppm ±20) and three levels of temperature (ambient, ambient+1.5°C and ambient+3.0°C) in 7.07m<sup>2</sup> OTC. The CO<sub>2</sub> concentration was maintained near 550 ppm during the daylight hours from 9:30 AM to 4:30 PM by

releasing compressed CO<sub>2</sub> gas from cylinders at the perimeter of the ring. It was supplied through tubes on the soil surface at 35cm height. The fluctuation of CO<sub>2</sub> concentrations in the OTC was 20 ppm. The temperatures of ambient+3.0 degrees and ambient + 1.5 degrees in OTCs were regulated by partial covering and not covering of the respective OTCs at the top by transparent Poly vinyl chloride sheets, respectively. Ambient temperature in the OTCs was maintained by making several perforations on side walls of the OTCs and making the gates open.

The crops were fertilized with 120, 26 and 50 kg ha<sup>-1</sup> N, P and K, respectively. All the P and K and 50% of N were applied at the time of sowing. The remaining N was applied in two equal splits at knee high (25 days after sowing, DAS) and tasseling (45 DAS) stages of maize.

Table 2. Treatment description.

| Sl. No. | Name | Description                                     |
|---------|------|-------------------------------------------------|
| T1      | T0C0 | Ambient temperature and ambient CO <sub>2</sub> |
| T2      | T1C0 | +1.5 °C & Ambient CO <sub>2</sub>               |
| T3      | T2C0 | +3.0 °C & Ambient CO <sub>2</sub>               |
| T4      | T0C1 | Ambient temp. & elevated CO <sub>2</sub>        |
| T5      | T1C1 | +1.5 °C & Elevated CO <sub>2</sub>              |
| T6      | T2C1 | +3.0 °C & Elevated CO <sub>2</sub>              |

## Growth parameters

The physiological data on net photosynthetic rate ( $P_N$ ), transpiration rate (Tr), intercellular CO<sub>2</sub> concentration (Ci), stomatal conductance (gs) and water use efficiency (WUE) were collected at tasseling and silking stages of maize crop. Gas-exchange measurements were taken on upper most fully expanded leaf using a portable Infra-Red Gas Analyser (IRGA) LICOR 6400 photosynthesis system (Li-Cor Inc. USA). The measurements were conducted between 9.30 to 11.0 AM on bright sunny days. Gas exchange parameters were analyzed at fixed light level by exposing the leaf to uniform PAR of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Throughout the experiment, gas flow rate to the console and analyzer was maintained at 400 mL min<sup>-1</sup>. Intact leaflet was clipped into leaf chamber and readings were logged after the leaf internal carbon dioxide (Ci) concentration became stable. Subsequently, net photosynthetic rate ( $P_N$ ), transpiration rate (Tr), intercellular CO<sub>2</sub> concentration (Ci) and stomatal conductance (gs) were recorded simultaneously. Intrinsic water use efficiency (WUE) was calculated from the ratio of  $P_N$  and Tr. Ten readings were taken for each

treatment at tasseling and silking stage of maize.

## Analysis of soil and plant samples

Soil samples were collected from 0-15cm depth using auger from three places and a composite sample from three places were analyzed for pH and EC (Jackson (1973), organic C (Walkey & Black (1934), dehydrogenase activity (DHA) (Casida *et al.* (1964), and available N (Subbiah & Asija (1956), P (Olsen *et al.* (1954), and K (Hanway & Heidal (1952) contents. Plant samples were also analyzed for crude protein and total N (Yoshida *et al.*, 1976), P and K contents (Jackson, 1973).

## Statistical analysis

Data were analyzed using SAS 9.3 statistical software for two way analysis of variance (ANOVA) to determine the effects of elevated CO<sub>2</sub>, temperature and their interactions on growth and yield parameters of maize. Statistical significance of the data were compared based on their 95% confidence intervals i.e.,  $P < 0.05$  (Gomez and Gomez, 1984).

## Results and Discussion

### Effect of Elevated CO<sub>2</sub> on Gaseous Exchange Parameters

Elevated CO<sub>2</sub> increased photosynthetic rate in both the years at tasseling and at first year of silking but decreased with same rate in second year at silking stage. Stomatal conductance and transpiration rate decreased in first year but increased in second year at tasseling but decreased in both the years at silking stage whereas intercellular CO<sub>2</sub> concentration increased in both the years at tasseling and decreased at silking stage. On the other hand, intrinsic water use efficiency increased in both the years at tasseling (Table 3) and silking stages (Table 4).

The mean values calculated for the two years data revealed that elevated CO<sub>2</sub> increased photosynthetic rate by 23.49%, stomatal conductance by 30.0% and intercellular CO<sub>2</sub> concentration by 59.80%, transpiration rate by 15.47% and intrinsic water use efficiency by 7.22% at tasseling stage of maize (Table 3). However, elevated CO<sub>2</sub> decreased photosynthetic rate by 1.14% and inter-cellular CO<sub>2</sub> concentration by 8.38% though there

was no significant effect, stomatal conductance by 30.16% and transpiration rate by 18.65% at silking stage of maize (Table 4). On the other hand, water use efficiency increased by 18.78% under elevated CO<sub>2</sub> because decrease in transpiration rate of plant was greater (18.78%) than photosynthetic rate (1.14%) at silking stage of maize compared to ambient CO<sub>2</sub> level (Table 5). Similar results have been found in line with the present finding. In line with this, some studies indicated that elevated CO<sub>2</sub> can increase plant gaseous exchange parameters like photosynthetic capacity and yield by adjusting its water state, so elevated CO<sub>2</sub> will have positive effect in water deficit condition (Leakey et al., 2006). A number of other controlled environment studies revealed that  $T_r$  decreased at elevated CO<sub>2</sub>. The decrease of  $T_r$  was associated with decrease of  $g_s$ , when elevated CO<sub>2</sub> decreased leaf  $g_s$ , and caused increasing resistance from intrinsic leaf to outside, resulting in the decrease of  $T_r$  of crops (Allen et al., 2011). Some controlled environment studies also showing that elevated CO<sub>2</sub> could cause a decrease in plant stomatal conductance and partly closing of the stomata (Ainsworth and Rogers, 2007).

Table 3. Effect of elevated CO<sub>2</sub> and temperature on leaf photosynthetic rate (P<sub>N</sub>), stomatal conductance (gs), intercellular CO<sub>2</sub> concentration (Ci), transpiration rate (Tr) and water use efficiency (WUE) of maize at tasseling stage.

| Treatment*  | P <sub>N</sub> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> ) |       |       | gs (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> ) |      |      | Ci (ppm) |        |        | Tr (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> ) |       |      | WUE (μmol CO <sub>2</sub> mmol H <sub>2</sub> O <sup>-1</sup> ) |      |      |
|-------------|------------------------------------------------------------------------|-------|-------|------------------------------------------------------------|------|------|----------|--------|--------|-------------------------------------------------------------|-------|------|-----------------------------------------------------------------|------|------|
|             | 2013                                                                   | 2014  | Mean  | 2013                                                       | 2014 | Mean | 2013     | 2014   | Mean   | 2013                                                        | 2014  | Mean | 2013                                                            | 2014 | Mean |
| T1          | 29.56                                                                  | 25.53 | 27.55 | 0.17                                                       | 0.16 | 0.16 | 143.61   | 88.71  | 116.16 | 3.08                                                        | 4.31  | 3.70 | 9.60                                                            | 5.93 | 7.77 |
| T2          | 30.45                                                                  | 28.69 | 29.57 | 0.26                                                       | 0.15 | 0.21 | 164.82   | 44.73  | 104.77 | 4.60                                                        | 5.29  | 4.95 | 6.63                                                            | 5.43 | 6.03 |
| T3          | 36.15                                                                  | 26.11 | 31.13 | 0.33                                                       | 0.13 | 0.23 | 216.18   | 32.91  | 124.54 | 7.49                                                        | 8.68  | 8.09 | 4.83                                                            | 3.01 | 3.92 |
| T4          | 33.95                                                                  | 39.76 | 36.85 | 0.20                                                       | 0.28 | 0.24 | 223.61   | 108.29 | 165.95 | 4.29                                                        | 8.76  | 6.52 | 7.95                                                            | 4.58 | 6.27 |
| T5          | 31.94                                                                  | 36.55 | 34.25 | 0.16                                                       | 0.32 | 0.24 | 193.99   | 166.68 | 180.33 | 3.44                                                        | 7.21  | 5.33 | 9.29                                                            | 5.08 | 7.19 |
| T6          | 32.39                                                                  | 43.37 | 37.88 | 0.21                                                       | 0.38 | 0.30 | 259.76   | 151.82 | 205.79 | 4.76                                                        | 10.21 | 7.48 | 6.83                                                            | 4.25 | 5.54 |
| SEM±        | 0.69                                                                   | 0.65  | 0.48  | 0.01                                                       | 0.01 | 0.01 | 13.05    | 11.10  | 4.88   | 0.13                                                        | 0.29  | 0.13 | 0.28                                                            | 0.24 | 0.12 |
| LSD(P=0.05) | 2.14                                                                   | 2.01  | 1.46  | 0.03                                                       | 0.03 | 0.02 | 40.21    | 34.21  | 15.04  | 0.39                                                        | 0.89  | 0.40 | 0.85                                                            | 0.73 | 0.36 |

\*Refer Table 2 for the description of the treatment

Table 4. Effect of elevated CO<sub>2</sub> and temperature on leaf photosynthetic rate (P<sub>N</sub>), stomatal conductance (gs), intercellular CO<sub>2</sub> concentration (Ci), transpiration rate (Tr) and water use efficiency (WUE) of maize at silking stage.

| Treatment*  | P <sub>N</sub> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> ) |       |       | gs (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> ) |      |      | Ci (ppm) |        |        | Tr (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> ) |      |      | WUE (μmol CO <sub>2</sub> mmol H <sub>2</sub> O <sup>-1</sup> ) |      |      |
|-------------|------------------------------------------------------------------------|-------|-------|------------------------------------------------------------|------|------|----------|--------|--------|-------------------------------------------------------------|------|------|-----------------------------------------------------------------|------|------|
|             | 2013                                                                   | 2014  | Mean  | 2013                                                       | 2014 | Mean | 2013     | 2014   | Mean   | 2013                                                        | 2014 | Mean | 2013                                                            | 2014 | Mean |
| T1          | 25.63                                                                  | 32.66 | 29.15 | 0.24                                                       | 0.23 | 0.23 | 174.06   | 124.65 | 149.36 | 7.39                                                        | 4.21 | 5.80 | 3.52                                                            | 7.75 | 5.63 |
| T2          | 27.93                                                                  | 30.93 | 29.43 | 0.29                                                       | 0.20 | 0.24 | 193.63   | 101.53 | 147.58 | 9.04                                                        | 3.63 | 6.34 | 3.10                                                            | 8.54 | 5.82 |
| T3          | 22.19                                                                  | 20.42 | 21.31 | 0.18                                                       | 0.14 | 0.16 | 168.65   | 131.20 | 149.93 | 6.35                                                        | 2.84 | 4.59 | 3.58                                                            | 7.28 | 5.43 |
| T4          | 27.39                                                                  | 21.55 | 24.47 | 0.20                                                       | 0.13 | 0.16 | 129.21   | 81.27  | 105.24 | 6.67                                                        | 2.65 | 4.66 | 4.12                                                            | 8.29 | 6.21 |
| T5          | 28.75                                                                  | 26.83 | 27.79 | 0.16                                                       | 0.14 | 0.15 | 213.82   | 52.62  | 133.22 | 5.85                                                        | 2.84 | 4.34 | 4.98                                                            | 9.51 | 7.24 |
| T6          | 32.34                                                                  | 21.09 | 26.72 | 0.19                                                       | 0.06 | 0.13 | 230.42   | 111.51 | 170.97 | 6.66                                                        | 2.55 | 4.61 | 4.93                                                            | 8.28 | 6.60 |
| SEM±        | 1.79                                                                   | 1.54  | 1.21  | 0.03                                                       | 0.01 | 0.02 | 23.12    | 20.71  | 14.65  | 0.65                                                        | 0.21 | 0.34 | 0.35                                                            | 0.53 | 0.33 |
| LSD(P=0.05) | 5.51                                                                   | 4.76  | 3.73  | 0.09                                                       | 0.03 | 0.05 | 71.25    | 63.81  | 45.13  | 2.01                                                        | 0.64 | 1.04 | 1.06                                                            | 1.63 | 1.02 |

Table 5. Mean individual effect of elevated CO<sub>2</sub> and temperature on leaf photosynthetic rate (P<sub>N</sub>), stomatal conductance (g<sub>s</sub>), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>), transpiration rate (Tr) and water use efficiency (WUE) of maize at tasseling and silking stages.

| Treatment                                | P <sub>N</sub><br>( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) |                 | g <sub>s</sub><br>( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) |         | C <sub>i</sub><br>(ppm) |                 | Tr<br>( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) |         | WUE<br>( $\mu\text{mol CO}_2$ |
|------------------------------------------|--------------------------------------------------------------------------|-----------------|----------------------------------------------------------------------|---------|-------------------------|-----------------|-----------------------------------------------------------|---------|-------------------------------|
|                                          | Tasseling                                                                | Silking         | Tasseling                                                            | Silking | Tasseling               | Silking         | Tasseling                                                 | Silking | Tasseling                     |
| <b>Effect of elevated CO<sub>2</sub></b> |                                                                          |                 |                                                                      |         |                         |                 |                                                           |         |                               |
| Mean ambient CO <sub>2</sub>             | 29.42                                                                    | 26.63           | 0.20                                                                 | 0.21    | 115.16                  | 148.96          | 5.58                                                      | 5.58    | 5.91                          |
| Mean elevated CO <sub>2</sub>            | 36.33                                                                    | 26.33           | 0.26                                                                 | 0.15    | 184.02                  | 136.48          | 6.44                                                      | 4.54    | 6.33                          |
| LSD (P=0.05)                             | 0.85                                                                     | NS <sup>a</sup> | 0.01                                                                 | 0.03    | 8.68                    | NS <sup>a</sup> | 0.23                                                      | 0.6     | 0.21                          |
| Effect of elevated CO <sub>2</sub> (%)   | 23.49                                                                    | -1.14           | 30.00                                                                | -30.16  | 59.80                   | -8.38           | 15.47                                                     | -18.65  | 7.22                          |
| <b>Effect of elevated temperature</b>    |                                                                          |                 |                                                                      |         |                         |                 |                                                           |         |                               |
| Mean ambient temperature                 | 32.20                                                                    | 26.81           | 0.20                                                                 | 0.20    | 141.06                  | 127.3           | 5.11                                                      | 5.23    | 7.02                          |
| Mean ambient temp. + 1.5°C               | 31.91                                                                    | 28.61           | 0.23                                                                 | 0.20    | 142.55                  | 140.4           | 5.14                                                      | 5.34    | 6.61                          |
| Mean ambient temp. +3.0°C                | 34.51                                                                    | 24.02           | 0.27                                                                 | 0.15    | 165.17                  | 160.45          | 7.785                                                     | 4.60    | 4.73                          |
| LSD (P=0.05)                             | 1.04                                                                     | 2.64            | 0.01                                                                 | 0.03    | 10.63                   | 31.92           | 0.29                                                      | 0.74    | 0.25                          |
| Effect of 1.5°C elevated temp (%)        | -0.90                                                                    | 6.71            | 12.50                                                                | 0.00    | 1.06                    | 10.29           | 0.59                                                      | 2.10    | -5.84                         |
| Effect of 3.0°C elevated temp (%)        | 7.16                                                                     | -10.43          | 32.50                                                                | -25.64  | 17.09                   | 26.04           | 52.35                                                     | -12.05  | -32.62                        |

<sup>a</sup>NS indicates non-significant at P<0.05

## Effect of Elevated temperature on Gaseous Exchange Parameters

Elevated temperature by 1.5 °C decreased photosynthetic rate at tasseling (Table 3) but increased at silking stage in both the years (Table 4). Stomatal conductance also increased at tasseling and silking stages. Intercellular CO<sub>2</sub> concentration decreased in the first year but had no effect on second year at tasseling stage where as Ci increased in the first year but decreased in the second year at silking stage. Intrinsic water use efficiency decreased in the first year but had no effect on second year at tasseling but increased both the years at silking stages with elevated temperature of 1.5°C. The mean values calculated for the two years data revealed that elevated temperature by 1.5 °C increased photosynthetic rate by 6.71% at silking stage, stomatal conductance by 12.5% at tasseling stage, intercellular CO<sub>2</sub> concentration by 1.06% and 10.29% both at tasseling and silking stages respectively, intrinsic water use efficiency by 10.3% at silking stage but decreased by 5.84% at tasseling stage. However, elevated temperature by 1.5 °C had no significant effect on photosynthetic rate, intercellular CO<sub>2</sub> concentration; transpiration rate at both tasseling and silking growing stages. Similarly, elevated temperature by 1.5 °C had no significant effect on stomatal conductance and intrinsic water use efficiency at silking stage as compared to ambient temperature (Table 5).

Further increase in temperature by 3.0 °C increased photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration and transpiration rate in both the years but decreased intrinsic water use efficiency in both the years at tasseling stage (Table 3). On the other hand, photosynthetic rate, stomatal conductance and transpiration rate decreased in both the years at silking stage. Similarly, intercellular CO<sub>2</sub> concentration increased in both the years and intrinsic water use efficiency increased in the first year but decreased in the second year at silking stage (Table 4). The mean values calculated for the two years data indicated that elevated temperature by 3.0 °C increased photosynthetic rate by 7.16%, stomatal conductance by 32.5% and intercellular CO<sub>2</sub> concentration by 17.09% and 26.0% at tasseling and silking stage respectively, transpiration rate by 52.35% at tasseling stage and intrinsic water use efficiency by 1.6% at silking stage. Elevated temperature by 3.0 degrees decreased photosynthetic rate by 10.43%, stomatal conductance by 25.64% and transpiration rate by 12.05% at silking stage and intrinsic water use efficiency by 32.62% at tasseling stage. Maximum increment of maize physiological parameter under elevated temperatures by 1.5 and 3.0 °C were observed in transpiration rate (52.35%) and stomatal conductance (32.5%) and maximum decrement was observed in intrinsic water use efficiency (32.62%). Elevated

temperature by 3.0 degrees had no significant effect on intrinsic water use efficiency at silking growing stage compared to ambient temperature level (Table 5).

### **Interaction Effect of elevated CO<sub>2</sub> and temperature on Gaseous Exchange Parameters**

Physiological gas exchange parameters in maize showed significant differences in the treatments under combined effect of elevated CO<sub>2</sub> and temperatures (ambient +1.5 and 3.0°C) except stomatal conductance, intercellular CO<sub>2</sub> concentration and intrinsic water use efficiency at silking stage in comparison to ambient temperature and CO<sub>2</sub> concentration (Table 4). Combined effect of elevated temperature by 1.5°C and 3.0 °C with elevated CO<sub>2</sub> concentration increased photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration and transpiration rate but decreased intrinsic water use efficiency in both the years at tasseling stage over plants under ambient CO<sub>2</sub> and temperature level. Similarly, increasing the temperature by 1.5 and 3.0 °C with CO<sub>2</sub> concentration increased photosynthetic rate and intercellular CO<sub>2</sub> concentration in the first year and intrinsic water use efficiency in both the years. Moreover, photosynthetic rate and intercellular CO<sub>2</sub> concentration in the second year, stomatal conductance and transpiration rate in both the years was decreased

under elevated temperature by 1.5°C and 3.0 °C with elevated CO<sub>2</sub> concentration at silking stage of maize compared to ambient CO<sub>2</sub> and temperature treatment. The mean values calculated for the two years data indicated that the interactive effects of elevated CO<sub>2</sub> with 1.5 and 3.0°C temperature rise increased photosynthetic rate by 24.32 & 37.5%, stomatal conductance by 50.0% & 87.5%, intercellular CO<sub>2</sub> concentration by 55.0% & 77.16%, transpiration rate by 44.0% & 102.16%, but decreased intrinsic water use efficiency by 7.46% & 28.7%, respectively at tasseling stage. On the contrary, elevated CO<sub>2</sub> with 1.5 and 3.0°C temperature rise decreased photosynthetic rate by 4.67 & 8.34%, stomatal conductance by 34.78% & 43.48%, transpiration rate by 25.17% & 20.52%, but increased intrinsic water use efficiency by 28.6% & 17.23%, respectively at silking stage. The interactive effects of elevated CO<sub>2</sub> with 1.5 °C temperature rise resulted in 10.81% decrease and elevated CO<sub>2</sub> with 3.0°C temperature rise resulted in a 14.47% increase in intercellular CO<sub>2</sub> concentration at silking stage as compared to ambient CO<sub>2</sub> and temperature. Elevated temperature by 1.5°C and 3.0 °C at elevated CO<sub>2</sub> had no effect on photosynthetic rate and intercellular CO<sub>2</sub> concentration. In addition, elevated temperature by 3.0 °C at elevated CO<sub>2</sub> had no effect on intrinsic water use efficiency at silking stage as compared to ambient CO<sub>2</sub> and temperature treatment (Table 5).

Similar results have been found in studies of some plant species which reported a significant increase in  $P_N$  under elevated  $CO_2$  and increased precipitation of 15% (Wang *et al.*, 2012). Wall *et al.* (2001) also observed that elevated  $CO_2$  increased  $P_N$  of sorghum by 9%. The decrease in photosynthesis during the high temperature treatment was less at elevated than at ambient  $CO_2$  concentrations consistent with expectations based on Rubisco thermal kinetics (Llod and Farquhar, 2008). The present study indicated that gaseous exchange parameters of maize improved with both elevated  $CO_2$  and temperature although the response of plant gaseous exchange parameters to each of the environmental variables like water availability, temperature, nitrogen along with the elevated  $CO_2$  has not been sufficiently understood (Lee, 2011).

## Conclusion

The mean values calculated for the two years data indicated that the interactive effects of elevated  $CO_2$  with 1.5 and 3.0°C temperature rise increased photosynthetic rate, stomatal conductance, intercellular  $CO_2$  concentration, transpiration rate at tasseling stage. On the contrary, elevated  $CO_2$  with 1.5 and 3.0°C temperature rise decreased photosynthetic rate, stomatal conductance, transpiration rate at silking stage. Elevated  $CO_2$  and temperature increased intrinsic water use efficiency at silking stage but

decreased at tasseling stage of maize. The effect of elevated  $CO_2$  and temperature on plants growth depends on the availability of other resources mainly nutrients and soil moisture. Hence, further study should be taken to ensure the contribution rate of elevated  $CO_2$  and temperature on different parameters of maize crop.

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# **Yields of Maize and Multiple Advantages of Pigeon Pea in Maize Intercropping at Bako, Western Ethiopia**

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## **Abstract**

Maize highly depletes nutrients in maize monocropping system and the situation is more serious when crop residues are continuously removed from the farmland. Consequently, the return from inorganic fertilizers application alone in the system is seasonal, requiring amendment of nutrients removed from the soil and retention of yield declined due to nutrient depletion. This experiment was executed at Bako in 2013, 2014 and 2015 main cropping seasons with the objective of evaluating the effect of branch removal of perennial pigeon pea and N level on yields of maize, on soil moisture and bulk density dynamics in maize/pigeon pea intercropping system. The treatments consisted of maize/pigeon pea intercropping in factorial combinations of four pigeon pea branch removals while leaving the upper (0, 2, 4 and 6) and five nitrogen levels (18, 41, 64, 87 and 110 kg ha<sup>-1</sup>) and sole maize monoculture laid out in randomized complete block design with three replications. Main effects due to pigeon pea branch removal and N level were significant for maize dry biomass yield during 2013 and 2014 and also significant for maize grain yield throughout the experimental periods. Removal of lower branches of pigeon pea while leaving the upper 2 in maize/pigeon pea intercropping increased grain yield of maize by 8% and produced higher dry biomass yield presumably due to taller maize plants which took relatively longer days to mature. Significantly increased soil moisture and reduced soil bulk density compared to the sole maize monoculture was obtained due to removal of lower branches of pigeon pea while leaving the upper 2 in maize/pigeon pea intercropping system. Higher dry biomass yield pigeon pea can be used as feed sources during feed shortage seasons. Hence, the system increased maize productivity at reduced N level, it is recommended for its maximization of crop and land productivity in the sub-humid maize based monocropping system of Bako and similar agro ecologies.

**Keywords:** Intercropping, maize, mono cropping, pigeon pea

## **Introduction**

Maize productivity in Ethiopia is currently increasing due to the release of many high yielding hybrid varieties. However, the productivity is low

compared to farmers' international potential yield. This yield gap is attributed mainly due to low soil fertility, crop diseases, insect pests and soil acidity resulted from maize monocropping. The system has been most common in the sub-humid areas

of western Ethiopia. CIMMYT (2010) reported that 94% of the respondent farmers from maize belt areas of Bako Tibe and Gobu Sayo districts grow maize continuously on the same plot of land. Nutrient depletion by maize in maize monocropping system is high and the situation is more serious when important plant nutrients are removed with crop residues. Consequently, the return from inorganic fertilizers application alone in the system is seasonal, requiring amendment of nutrients removed from the soil and retention of yield declined due to nutrient depletion.

The tradition of leaving degraded farm lands fallow but practice open livestock grazing aiming at improving soil fertility lead to the lowest organic matter contribution compared to the mixed crop lands (Asif *et al.*, 2014). Declining of soil fertility aggravated by wide scale continuous cultivation of maize which mines the soil nutrients remained the farmers struggling to maintain yields year after year (Snapp *et al.*, 2010). Rising human consumption from crop source is dramatically increasing depends on agriculture and natural resources which raises challenges for achieving food security (Foley *et al.*, 2011). Through strategic improved crop varieties and inorganic fertilizer use, it might not be enough to feed the growing population requiring production of more food on the same amount of land (Giller *et al.*, 2006).

Cereal/legume cropping system show considerable promise in boosting

productivity, helping reverse the decline in soil fertility (Dagne *et al.*, 2012) and improves dietary sources. Incorporating nitrogen fixing legumes in to maize based cropping systems has the potential to improve soil fertility and mitigate the nutrient mining of maize (Snapp and Silim, 2002). Pigeon pea in the systems with advantages of fertilizer value, dry season animal feed and seeds for human consumption (Abebe and Diriba, 2002) has the potential of reacting with iron-bond phosphate in the soil to release P (Hector and Smith, 2007).

Ethiopia with wide range of ecological suitability to pigeon pea didn't widely enter into the multiple uses from the crop. Zerihun *et al.*(2016) reported perennial pigeon pea can be used as a live-stake for climbing bean and production of finger millet as intercrop during the establishment phase. The litter fall reduces soil erosion, improves soil fertility and larger root mass contributes to improvement of soil organic matter. Faster establishment in subsequent years reduces cultivation cost when conservational agriculture is used. Therefore, the objective of this study was to evaluate the effect of branch removal of perennial pigeon pea and N level on crop yields of maize, on soil moisture and bulk density dynamics in maize/pigeon pea intercropping system at maize belt areas of Bako, western Ethiopia.

## **Materials and Methods**

### **Description of the study area**

The experiment was conducted on reddish-brown clay-loam Nitisols in 2013, 2014 and 2015 cropping season at Bako Agricultural Research Center, which is situated in the western part of Ethiopia at an altitude of 1650 m.a.s.l. The area has a warm-humid climate with annual minimum and maximum temperatures of 13.6 and 29.1<sup>0</sup> C, respectively. Long-term average annual rainfall of the area is 1264 mm.

### **Planting materials**

Maize variety (BH-661) which is relatively suitable for the intercropping system due to its semi-erect morphology and stay green after maturity and perennial multi-purpose pigeon pea locally adapted to the area were used for the study.

### **Experimental design and procedure**

Twenty one treatments consisted of factorial combinations of four branch removals (pruning) but leaving 0, 2, 4 and 6 upper branches of pigeon pea and five nitrogen levels (18, 41, 64, 87 and 110 kg N ha<sup>-1</sup>) as well as sole maize with recommended NP were used in a randomized complete block design with three replications. The land was prepared using tractor implement during the initial year (2013) whereas crop residue incorporation and minimum tillage using local digging hoe was used

during the second (2014) and third (2015) years of the experiment. Maize was planted at a spacing of 75 cm x 30 cm on a plot area of 19.125m<sup>2</sup> (3.75m x 5.1m). Pigeon pea was also planted between rows of maize at 50cm spacing between plants and branches were removed 8 weeks after planting. During 2014 and 2015 cropping seasons, branches were removed right at planting of maize according to the treatment set up. The removed branches were weighed (biomass weight in this context), chopped and incorporated to the soil of respective plots. Data such as grain and biomass yields for both crops were collected from three center rows.

The composite soil sample was collected from each experimental plot at depth of 0-20 cm before planting (at establishment) and after harvest of each cropping season. Soil samples were weighed and oven dried at 105<sup>0</sup>. Samples were analyzed for moisture content and bulk density determinations.

### **Data collection and management**

Grain yield was corrected to 12.5% and 10% moisture content standards for maize and pigeon pea, respectively. Above ground biomass of maize and sample biomass of pigeon pea (pruned branches with leaves) were sun-dried until constant weight was maintained.

### **Data analysis**

Data were analyzed using SAS version 9.1 (SAS, 2008) computer software

and were subjected to ANOVA to determine significant differences among factors and their interactions. Means were separated using LSD test (Steel and Torrie, 1981).

## Results and Discussion

### Biomass and grain yields of maize

Mean maize grain yield obtained from maize/pigeon pea intercropping regardless of pruning options resulted in 6% yield advantage over maize grown sole during the initial establishment of pigeon pea, 2013 main cropping season. Combined maize grain yield, advantage of 1.25% was recorded from maize pigeon pea intercropping compared to the maize monoculture (Table 1).

The main effect due to branch removal of pigeon pea was significant for maize biomass and grain yields. Removing pigeon pea branches but leaving 0 and 2 upper ones produced significantly the highest maize grain yields during the three cropping seasons (Table 1). Indeed, mean maize grain yields of 8940 kg ha<sup>-1</sup> and 8884 kg ha<sup>-1</sup> were recorded for branch removals leaving 0 and 2 upper ones, respectively against 8471 kg ha<sup>-1</sup> obtained by maize grown sole with recommended fertilizer of 110 46 kg ha<sup>-1</sup> NP<sub>2</sub>O<sub>5</sub> (Table 1). Higher maize grain yield in maize/pigeon pea intercropping might have attributed by less competition, addition of organic matter to the soil and improvement of soil moisture. Removal of lower

branches but leaving 6 upper ones produced significantly lowest maize grain yield throughout the cropping seasons (Table 1) which presumably due to high shading effect and competition for resources. Likewise Daniel and Ong (1990) confirmed perennial pigeon pea intercropped with sorghum has improved yield of component crop when lower branches were removed. Unaffected grain yield of component crop was also reported by ICRISAT (1987) when plant population of pigeon pea was reduced below 28000 and lower branches were removed.

The main effect of nitrogen level was affected maize biomass and grain yield during the three cropping seasons (Table 1). Application of 110 kg N ha<sup>-1</sup> and 87 kg N ha<sup>-1</sup> produced significantly higher maize grain yields during the three cropping seasons (Table 1). Application of 18 kg ha<sup>-1</sup> produced significantly lower maize grain yield throughout the three cropping seasons, but not significantly different from application of 41 kg N ha<sup>-1</sup> during 2013 and 2015 cropping seasons.

### Biomass and grain yields of pigeon pea

Biomass weight of pigeon pea increased as the rate of pigeon pea branch removal increased throughout the cropping seasons and showed increasing trend for subsequent cropping seasons (Table 2). Pruning of all branches but leaving the growing meristem produced significantly

higher biomass weight of 8299 , 8546 and 9255 kg ha<sup>-1</sup> but not significantly different from removing all branches but leaving 2 upper ones during 2013, 2014 and 2015, respectively (Table 2). Mean biomass weight for removal of all lower branches leaving the upper 6 and 4 were significantly the lowest during the three cropping seasons.

Unlike the biomass weight, seed yield increased as the rate of branch removal decreased throughout the cropping seasons. The main effect of branch removal affected seed yield of pigeon pea throughout the cropping seasons. Certainly, significantly higher seed

yields of 1234 , 1339 and 1441 kg ha<sup>-1</sup> during 2013, 2014 and 2015, respectively were obtained from branch removal but leaving 6 upper ones. All branches removal leaving only the upper meristem produced significantly the lowest seed yields, but as high as 807 kg ha<sup>-1</sup> during 2015 cropping, considerably additional output obtained from rejuvenated branches during off-seasons (Table 2). Fast re-growth habit of branches after pruning and the time interval from pruning to the crop phenology favored annual seed harvest from perennial pigeon pea.

Table 1. Maize biomass and grain yield as affected by the main effects of pigeon pea branch removal and N level in maize/pigeon pea intercropping at Bako

| Factor                                    | Biomass weight (kg ha <sup>-1</sup> ) |                     |                     | Grain yield (kg ha <sup>-1</sup> ) |                   |                    |
|-------------------------------------------|---------------------------------------|---------------------|---------------------|------------------------------------|-------------------|--------------------|
|                                           | 2013                                  | 2014                | 2015                | 2013                               | 2014              | 2015               |
| Pigeon pea branch removal & leaving upper |                                       |                     |                     |                                    |                   |                    |
| 0                                         | 24677 <sup>a</sup>                    | 22501 <sup>a</sup>  | 19547               | 9987 <sup>a</sup>                  | 8602 <sup>a</sup> | 8232 <sup>a</sup>  |
| 2                                         | 23822 <sup>ab</sup>                   | 21347 <sup>ab</sup> | 19616               | 9822 <sup>a</sup>                  | 8569 <sup>a</sup> | 8260 <sup>a</sup>  |
| 4                                         | 23189 <sup>ab</sup>                   | 20054 <sup>bc</sup> | 19891               | 9523 <sup>ab</sup>                 | 7933 <sup>b</sup> | 7897 <sup>ab</sup> |
| 6                                         | 22646 <sup>b</sup>                    | 19376 <sup>c</sup>  | 20031               | 9011 <sup>b</sup>                  | 7658 <sup>b</sup> | 7429 <sup>b</sup>  |
| SE ±                                      | 637                                   | 210                 | 547                 | 456                                | 109               | 221                |
| LSD (5%)                                  | 1819                                  | 1303                | NS                  | 599                                | 312               | 632                |
| Sole maize                                | 22411                                 | 18767               | 21363               | 9040                               | 8193              | 8179               |
| N level (kg ha <sup>-1</sup> )            |                                       |                     |                     |                                    |                   |                    |
| 18                                        | 21749 <sup>b</sup>                    | 18783 <sup>b</sup>  | 17017 <sup>c</sup>  | 8426 <sup>b</sup>                  | 7015 <sup>c</sup> | 6819 <sup>c</sup>  |
| 41                                        | 24124 <sup>a</sup>                    | 20959 <sup>a</sup>  | 17953 <sup>c</sup>  | 9017 <sup>b</sup>                  | 8243 <sup>b</sup> | 7158 <sup>c</sup>  |
| 64                                        | 23654 <sup>ab</sup>                   | 20827 <sup>a</sup>  | 20041 <sup>b</sup>  | 10071 <sup>a</sup>                 | 8145 <sup>b</sup> | 8132 <sup>b</sup>  |
| 87                                        | 24366 <sup>a</sup>                    | 21623 <sup>a</sup>  | 21340 <sup>ab</sup> | 10205 <sup>a</sup>                 | 8730 <sup>a</sup> | 8671 <sup>ab</sup> |
| 110                                       | 2406 <sup>a</sup>                     | 21907 <sup>a</sup>  | 22505 <sup>a</sup>  | 10209 <sup>a</sup>                 | 8818 <sup>a</sup> | 8995 <sup>a</sup>  |
| SE ±                                      | 712                                   | 234                 | 612                 | 510                                | 122               | 247                |
| LSD (5%)                                  | 2034                                  | 1457                | 1750                | 669                                | 349               | 707                |
| CV (%)                                    | 10.45                                 | 8.49                | 10.78               | 8.47                               | 5.17              | 10.72              |

Table 2. Biomass and seed yield of pigeon pea as affected by the main effect of pigeon pea branch removal in maize/pigeon pea intercropping at Bako

| Factor                                  | Branch weight kg ha <sup>-1</sup> |                    |                    | Seed yield kg ha <sup>-1</sup> |                   |                   |
|-----------------------------------------|-----------------------------------|--------------------|--------------------|--------------------------------|-------------------|-------------------|
| Pigeon pea branch removal leaving upper | 2013                              | 2014               | 2015               | 2013                           | 2014              | 2015              |
| 0                                       | 8299 <sup>a</sup>                 | 8546 <sup>a</sup>  | 9225 <sup>a</sup>  | 721 <sup>d</sup>               | 747 <sup>c</sup>  | 807 <sup>c</sup>  |
| 2                                       | 7016 <sup>ab</sup>                | 7238 <sup>ab</sup> | 7813 <sup>ab</sup> | 836 <sup>c</sup>               | 1001 <sup>b</sup> | 1079 <sup>b</sup> |
| 4                                       | 6937 <sup>b</sup>                 | 7197 <sup>b</sup>  | 7642 <sup>b</sup>  | 931 <sup>b</sup>               | 951 <sup>b</sup>  | 1010 <sup>b</sup> |
| 6                                       | 5763 <sup>b</sup>                 | 5910 <sup>b</sup>  | 6360 <sup>b</sup>  | 1234 <sup>a</sup>              | 1339 <sup>a</sup> | 1441 <sup>a</sup> |
| SE ±                                    | 471.29                            | 466.57             | 512.39             | 31.76                          | 37.16             | 41.87             |
| LSD (5%)                                | 1346                              | 1334               | 1465               | 88                             | 106               | 120               |
| CV %                                    | 12.88                             | 25.02              | 25.57              | 26.04                          | 14.25             | 14.96             |

### Soil moisture

Soil moisture ranged between 12.5 and 17.8 % being the lowest for maize monocropping and the highest for maize/pigeon pea intercropping while pruning all lower branches but leaving 2 and 0 upper branches (Fig. 1A). Despite of pigeon pea biomass and maize crop residue retention, both the intercropped and sole maize monoculture plots showed increasing trend (Fig. 1A) throughout the three cropping seasons. Indeed, maize/pigeon pea attained suitable moisture content for maize growth with respect to the soil type of the Bako area. Similarly, Karuku *et al.* (2014) reported that incorporation of vetch crop residue influenced water content, water use efficiency and increased tomato yield.

### Soil bulk density

Bulk density remained above 1.2 for sole maize continuous cropping plots whereby maize/pigeon pea intercropping showed remarkable improvement, maintained below 1.15 for pruning all lower branches but leaving the upper 2 and the growing meristem (Fig. 1B). The wider range between plots of

maize/pigeon pea intercrop and maize sole could be due to large and easily degradable deposit of pruned and incorporated pigeon pea branches resulted from high organic matter build up. Similarly, Shaver (2010) justified crop residue is lighter than mineral matter, promoted more aggregation and increased root activity that favored aggregation as a result reduced bulk density.

### Effective Rainfall Use Efficiency (ERUE)

The rainfall received during 2013, 2014 and 2015 cropping seasons was 1431, 1067 and 944 mm, respectively (Fig. 2B). With similar decreasing trend, effective rainfall during the crop growing season was 1227, 830 and 779 mm during 2013, 2014 and 2015, respectively (Fig. 2B). Maize ERUE progressively increased from 2013 to 2015 cropping season for all pigeon pea branch removals and for sole maize (crop residue maintained in this case). During 2013 and 2014 cropping seasons, the highest ERUE of 8 and 10 kg ha<sup>-1</sup> mm<sup>-1</sup>, respectively were recorded by maize intercropped with pigeon pea removed all lower branches while leaving 2 and 0 upper

branches. During 2015, maize intercropped with pigeon pea with respect to all branch management and sole maize produced grain over 10 kg ha<sup>-1</sup> mm<sup>-1</sup> (Fig. 2A) which could be

attributed due to both pigeon pea branch and maize crop residue maintenance on each experimental plot.

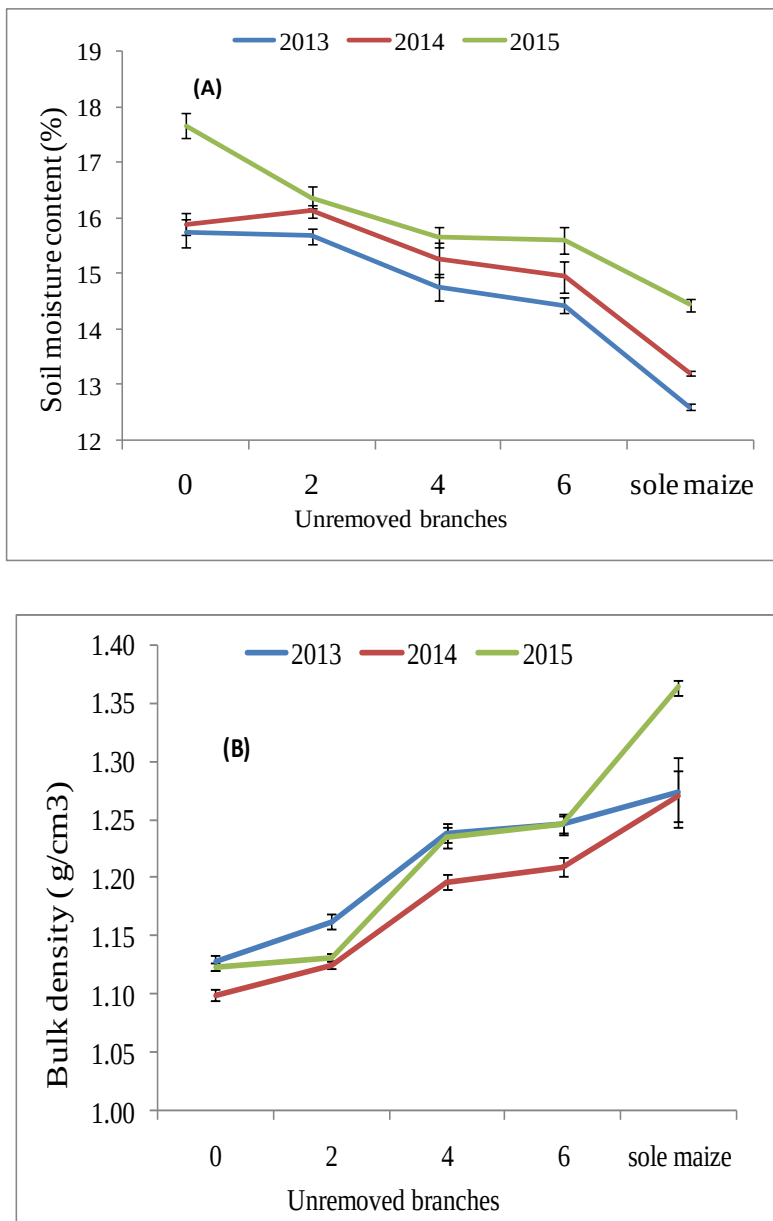


Figure 1: (A) soil moisture and 1(B) bulk density as affected by branch removal of pigeon pea in maize/pigeon pea intercropping at Bako during 2013, 2014 and 2015 cropping seasons

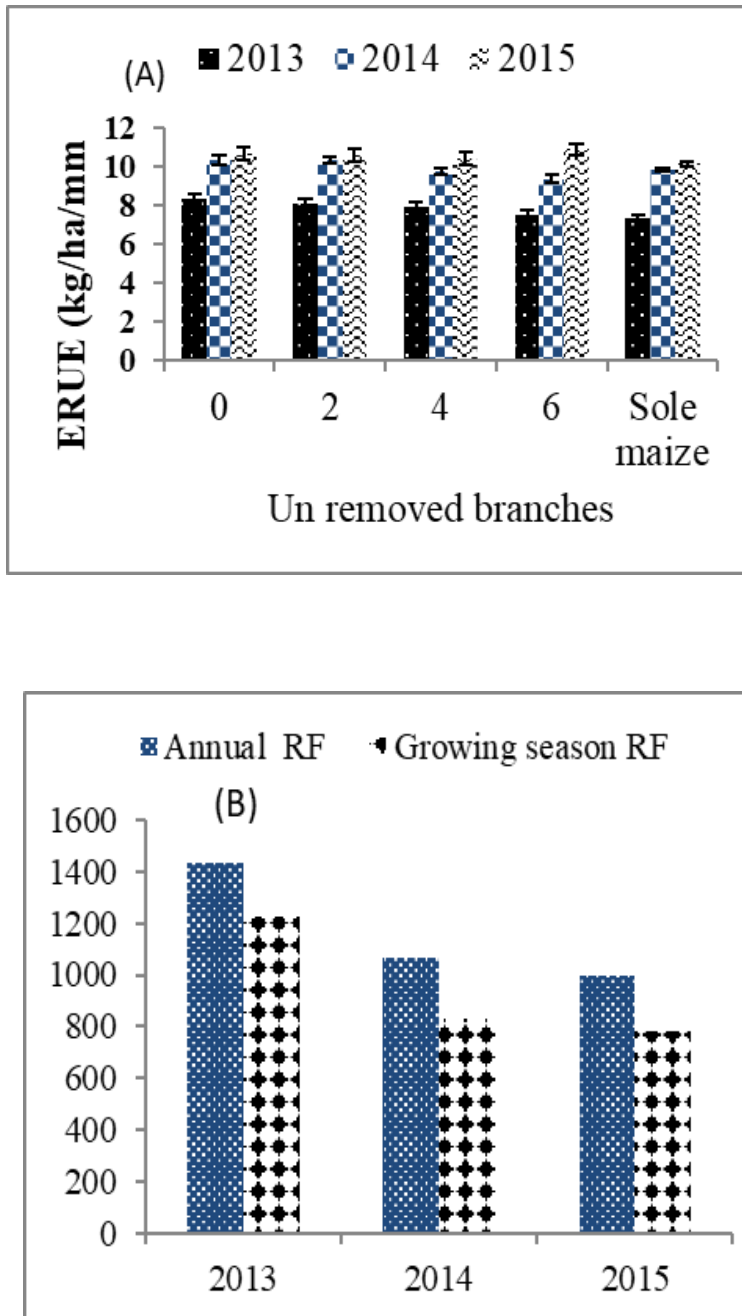


Figure 2: (A) ERUE as affected by branch removal of pigeon pea in maize/pigeon pea intercropping and (2B) Annual rainfall and growing season rainfall at Bako during 2013, 2014 and 2015 cropping seasons

## Conclusion

In maize belt areas of western Ethiopia, larger number of hybrid varieties with high yielding potentials is released by research. Obviously, the potential of the crop and land productivity could decrease due to continuous cultivation of the same crop. It is also very difficult to put single technological solutions for multiple productivity threats of the area because important plant nutrients removed with crop residues cannot be replaced from inorganic fertilizer alone. Pigeon pea is an important crop with great potential to reverse the soil fertility decline and improves the yield potential of component crop. The large biomass obtained from pigeon pea improved soil moisture and soil bulk density resulted from increased organic matter whereby competition for light was managed by branch removal and competition for moisture was compensated by the high rain fall distribution in the area and high organic matter restoration from incorporation of pigeon pea biomass to the soil. Seed obtained from pigeon pea is a good source of supplementary digestible protein and starch source for human diet and identified as chicken feed. Grain and biomass yield of maize and maize ERUE was increased at less annual rainfall and less growing season rainfall whereby the maintained biomass improved moisture availability following cessation of rainfall. Maize variety, BH 661 has stay green stem with elongated maturity period in maize/pigeon/pea

intercropping so that the integrated biomass prevented the soil from fast drying. Thus, incorporation of perennial pigeon pea to the maize dominated cropping system requires due attention by research and extension system.

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# Effect of Altitude, Shade, and Processing Methods on Isotope and Biochemical Composition of Green Coffee Beans in Ethiopia

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## Abstract

Though Arabica coffee has evolved in Ethiopian natural forests, it is being produced without shade in many countries at varying altitudes. Thus, the objective of this study was to determine the composition, fingerprinting, and association of isotopes and biochemical composition as a function of altitude, shade, and processing methods. Carbon, nitrogen and oxygen Isotopes as well as %N and %C of the beans were determined using a continuous flow (CF) EA-IRMS, while caffeine, trigonelline and chlorogenic acid content were determined using HPLC/THERMO. Sucrose content was determined using Gas Chromatography. The study included washed and unwashed coffee samples from altitudes of 1150, 1545 and 1802 meters above sea level. For the determination of the aforementioned components of the bean, univariate analysis of variance, automatic regression modelling, and stepwise canonical discriminant functions were used. Percent N,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and %C did not show significant result by altitude. For  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ , unshaded coffee showed higher mean value than the shaded counterpart. Shading disfavoured the composition of isotopic oxygen ( $\delta^{18}\text{O}$ ) as the altitude increases. Shaded coffee at 1150 meters above sea level (m a.s.l.) showed the highest mean value while at 1802 m a.s.l. the lowest for both sucrose and trigonelline composition of green coffee beans. Washed coffee at 1802 m a.s.l. for 5-CQA, unwashed coffee at 1150 m a.s.l. for CFQA, unwashed coffee at 1545 m a.s.l. for TCGA, unwashed coffee at 1150 m a.s.l. for both sucrose and trigonelline showed the highest mean value while unwashed coffee at 1802 m a.s.l. for 5-CQA, TCGA, sucrose and trigonelline, and washed coffee at 1545 m a.s.l. for CFQA showed the least value. The highest mean value was obtained from unwashed-shaded coffee at 1545 m a.s.l. while unwashed- shaded coffee at 1802 was the lowest. The association of isotopes and biochemical composition of green coffee beans was found to be weak although for caffeine and percent N contributed a significant positive weight ( $b = 6.604$ ,  $P \leq 0.002$ ) and %C contributed a significant negative ( $b = -0.388$ ,  $P \leq 0.004$ ) weight for the model. Lowland coffees were well discriminated (94.4% of variation) by 4,5-Dicaffeoylquinic acids (4,5-DCQA) compared to coffees from midlands and highlands.

**Keywords:** Caffeine, Chlorogenic acids, Coffee quality profile, Coffee value chain, finger printing,

## Introduction

Globalization of the coffee market increasingly concerns labelling of the origin of the produce. It is understood coffee quality has a strong association with its origin. Several attempts have been made to discriminate analytically the origin of green and roasted coffees. Signatures (finger printings) of various natures could be used for sorting the products of unique nature (Meinzer *et al.*, 1990). A review by Rodrigues *et al.* (2009) discloses different approaches of identifying origins of food stuffs for labelling. Analytical methods such as gas chromatography–mass spectrometry (Costa Freitas *et al.*, 2001), near infrared spectroscopy (Bertrand *et al.*, 2005), determination of organic compounds such as chlorogenic acids and fatty acid profiles (Martí'n *et al.*, 2001), tocopherols and triglycerides (González *et al.*, 2001) and stable isotope analysis of specific compounds extracted from the green coffee beans (Adugnaw, 2014) have used extensively with promising results.

Ethiopia, despite being the place of origin and diversification of Arabica coffee, has not yet fully exploited its genetic and environmental wealth discriminated in the world market (Petit, 2007). The country could play important roles as a supplier of some of the best coffees in the 'global coffee value chain', which could be accompanied by discrimination and promotional work that could identify it with unique inherent flavour. In this

regard, the approach using stable isotope ratios and biochemical composition for origin discrimination seem quite promising. Serra *et al.* (2005) inferred the continental origin (Africa, Asia and America) of coffee using the combination of the isotopic fingerprints of carbon, nitrogen and boron, which are reportedly used as integrated proxies for environmental conditions and agricultural practices. The authors were successful in identifying 88% of the intercontinental origin of the samples but not an intracontinental discrimination by means of principal component analysis. Variation in stable carbon isotope discrimination among genotypes grown under identical conditions has been used as a means of selecting for improved water use efficiency (WUE) and yield in  $C_3$  crop species (Meinzer, *et al.*, 1990).

Environmental factors, such as altitude, shade and rainfall may contribute to the quality of the coffee beverage (Bote and Struik, 2011; Likassa Ebisa, 2014). However, further studies are needed to investigate additional environmental characteristics that affect coffee quality (Rodrigues *et al.*, 2009) reported that an evaluation of the caffeine content of beans from 99 progenies revealed intra- and inter-progeny variability. In 68 progenies from the Kaffa region they found caffeine values in the range 0.46–2.82%, and in 22 progenies from Illubabor region these values ranged from 0.42 to 2.90%. Bertrand *et al.*

(2006) reported a significant effect of Elevation on bean biochemical composition of the cultivar 'Caturra' where they found increased concentration of chlorogenic acid and caffeine with increasing elevation above 1100 m. The reverse trend was observed for trigonelline concentration in one situation and had no effect on another. Caffeine and fat concentrations increased with increasing elevation up to a certain level.

Natural diversity in Ethiopian specialty coffees may represent an important opportunity to add value to the economic and social development of the country as well as to the advancement of coffee science. Systematically establishing a relationship between geographic origin of coffee and its quality leading to the generation of reliable data is a priority area in Ethiopia (Adugnaw, 2014). However, studies on specialty coffee discrimination and authentication methods have been only recent phenomenon in Ethiopia. Such methods could complement the existing subjective organoleptic classifications methods. Thus, the objective of this study was to describe the composition, fingerprinting, and association of coffee bean isotopes and biochemical compounds as a function of altitude, shade and processing methods.

## **Materials and Methods**

### **Site selection and experimental design**

The study included washed coffee and unwashed coffee samples originating from south-western Ethiopia from three farms located at different altitudes (1150, 1545 and 1802 metres above sea level designated as lowland, midland and highland coffee growing areas, respectively (MoA, 2003). According to the data obtained from National Meteorological Services Agency (2010) the region is characterized by monomodal rainfall of about 1565 mm, maximum and minimum temperatures of 26.1 and 13.2 °C, respectively, relative humidity of 73.3%, sunshine hours of 5.4 and altitude ranging between 1150 and 1820 meters above sea level. The farms were purposely selected considering the altitudinal ranges and uniformity to management methods. The selected farms belonged to Coffee Plantation Development Enterprise. Nine sub coffee farms were selected from the three farms. From the three coffee farms, shaded and unshaded plots were identified and two types of processing methods were applied in three replications. Ripe coffee cherries were handpicked at their peak ripening phases during the 2010/11 crop season out of which washed and unwashed green coffee beans were carefully prepared without contamination.

Table 1. Description of study sites

| Farm          | Region         | Latitude    | Longitude    | Altitude (m a.s.l.) |
|---------------|----------------|-------------|--------------|---------------------|
| Bebeka (N= 3) | Benchmaj/ SNNP | 6°56.580' N | 35°30.607' E | 1150                |
| Kossa (N= 3)  | Jimma/ Oromia  | 7°57.223' N | 36°52.664' E | 1802                |
| Goma (N= 3)   | Jimma/ Oromia  | 7°55.253' N | 36°37.069' E | 1545                |

## Laboratory analysis

Green coffee beans were subjected to freeze drying just before grinding to fine powder using a hand-held electrical Blade coffee grinder (Bosch MKM 6003 UC, Bean Container Capacity: 75g, Power: 180 Watt). Grinding was assumed to be sufficient when the powder escaped to the ceiling of the cap of the grinder, and it was immediately packed in a plastic cup with a tight stopper, and kept in a deep freezer until laboratory analysis.

## Carbon, Nitrogen isotopes, and elements measurement

Carbon and Nitrogen Isotopes and %N and %C were measured using a continuous flow (CF) EA-IRMS, Sercon stable isotope mass spectrometers (UK) (OTSUKI, 1983). Finely ground green coffee bean powder (0.95 - 1.4 mg each) rolled in small tin capsules were loaded onto an auto sampler in a duplicate. The samples were purged by a helium (He) flow into a combustion tube and completely oxidized at a temperature of 1000 °C. A packed GC column removes impurities and separates N<sub>2</sub> and CO<sub>2</sub>. A mass spectrometer ionizes gaseous molecules and separates the ions into a spectrum according to their mass-to-charge ratio (m/z), using electric and magnetic fields. The

relative abundances of the molecules of different m/z were then found by measuring the currents generated by these separated ion beams. A high vacuum keeps the analyzer pressure low enough (10-5 mbar) to reduce collisions between ions and background gas to an acceptable level. A permanent magnetic field was used (fixed B) and masses were selected by varying the tensions of the electric field V. A universal triple collector was used and B and V were kept constant for each element that has to be measured (Otsuki, 1983).

## Oxygen (δ18O) isotope measurement (VSMOW)

Oxygen isotope measurement was done via a TC/EA (thermal conversion/elemental analyzer) coupled to an isotope ratio mass spectrometer (IRMS) (20-20, SerCon Ltd, Crewe, UK) (SerCon, 1983.). The green coffee bean powder was further dried overnight at 60-80 °C and stored in a desiccator after which each of a 1 mg samples was rolled in a silver cup in a duplicate and loaded onto an auto sampler. The samples were pyrolyzed at 1400 °C in a molybdenum lined, aluminium oxide reduction tube filled with glassy carbon and topped with a glassy carbon crucible. The produced N<sub>2</sub> and

CO gases were separated via a 1 m gas chromatography (GC) column (E3030, Elemental Microanalysis Ltd., Okehampton, UK) at a temperature of 50 °C, helium (He) carrier gas pressure of 1.6 bar, Helium (He) flow retention time of about 250 s, and sample analysis time of 1000 s, and analyzed via isotope ratio mass spectrometry (IRMS) for  $\delta^{18}\text{O}$ . The internationally accepted reference values of  $\delta^{18}\text{O}$ -KNO<sub>3</sub> for USGS32 (25.7±0.4 and USGS34 (-27.8±0.4, (Brand *et al.*, 2009) and  $\delta^{18}\text{O}$ -NaNO<sub>3</sub> for USGS35 (56.8±0.3 (IAEA, 2004) were used to correct raw  $\delta^{18}\text{O}$  values to  $\delta^{18}\text{O}$  (‰) (Otsuki, 1983).

### **Caffeine, Trigonelline and Chlorogenic acids**

The caffeine and chlorogenic acid contents were determined using HPLC/THERMO following the method of Alonso-Salces *et al.* (2009): 0.1 g freeze dried green coffee powder was weighed in an Erlenmeyer flask of 50 ml. 10 ml of MeOH/Acetic Acid (30:7.5:2.5) containing 2 mg/ml ascorbic acid was added and then placed in an ultrasonic bath for 15 minutes. The extract was filtered using Whatman filter papers No.2, and subsequently over a 0.45 micrometer PTFE filter after which 1 ml of the filtrate was taken in a vial and injected on HPLC/THERMO. The standard solutions of chlorogenic acid, caffeine, and caffeic acid were mixed each at 0.5/1/1.5 mg/ml in one mixture in methanol and each solution was injected twice for calibration. A calibration curve was made using the

standard concentration and area of sample and subsequently used to calculate the composition of the respective biochemical component using the area generated after the retention time. The detection was carried out at 278 nm for caffeine and trigonelline, and 324 nm for chlorogenic acids (CGAs). For the identification and quantitative analysis, a standard curve was prepared using standards of caffeine, trigonelline and Chlorogenic acids.

### **Sucrose measurement**

Sucrose of the coffee beans was determined using GC VARIAN 3800 following the standard method. A sample of green coffee powder was freeze-dried and weighed (0.5 - 1 g) in 50 ml volumetric flask to which 30 ml distilled water plus 5 ml frozen Internal Standard Solution (ISS) (Phenyl-B-D-pyranoside) was added. It was placed at 60 °C for 30 minutes after which it was cooled. Next, 0.5 ml each of Carre I (15 g ZnSO<sub>4</sub> and 7.5 g Carre II (K<sub>4</sub>Fe(CN)<sub>6</sub>) was added to de-protein the sample. The distilled water was then filled to label of the mark on the 250 volumetric flasks and shaken well to homogenize the mixture. The solution was immediately filtered with Whatman filter papers, and subsequently 1 ml filtrate was taken in small bottles using glass Pasteur pipette and dried under nitrogen-drier using hollow needles to let nitrogen in to the bottle. To this dry extract was added 1 ml STOX (2.5 g hydroxylamine hydrochloride diluted with dry pyridine to 100 ml) under

hood and kept at 60 °C for 30 minutes and then cooled down. Then, 1 ml of HMDS (hexamethyldisilazan) was added and subsequently 0.1 ml TFA (trifluor acetic acid) was added before sedimentation for 60 minutes to get clear extract solution. From the clear extract solution, 1 ml was taken in a vial with rubber stopper to inject to GC VARIAN 3800.

### Statistical analysis

Univariate Analysis of Variance and Tukey HSD method of mean separation was applied to study the significant difference. Moreover, multiple regression analyses were conducted to examine the relationship between coffee quality attributes and various potential predictors using SPSS 16 v2 software (SPSS Inc.2007). Stepwise canonical discriminant function and Stepwise Regression between isotopes and biochemical composition of green coffee beans were conducted to identify the best predictor for discriminating coffees of different altitudinal gradients.

## Results and Discussion

### Isotopic and Percent N and C composition of green coffee beans as a function of altitude, shade, and processing methods

This study showed that there were significant effects ( $P \leq 0.05$ ) of altitude on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Shade also showed significant effect on  $\delta^{13}\text{C}$ , and

non-significant, otherwise ( $p > 0.05$ ) (Table 2-4 and Figure 1). With regard to  $\delta^{15}\text{N}$ , the mean value at 1802 meters above sea level was significantly lower than the mean value at both 1150 and 1545 meters above sea level. The trend showed that  $\delta^{15}\text{N}$  concentration increased in the order of lowland, midland and highland samples (Figure 1). The mean value of  $\delta^{15}\text{N}$  at 1802 meters above sea level was significantly lower as compared to the mean value at both 1150 and 1545 meters above sea level likely due to lower microbial deposition and hence discrimination during the decomposition process of N at higher altitudes. Most elements of biological interest have two or more stable isotopes, although one isotope is usually present in far greater abundance (Ehleringer and Rundel, 1989). While there is no evidence for biological fractionation of the isotopes, they may be potentially useful markers of ecosystem process studies.

One of the factors controlling the mineral content of plant material is the availability of plant nutrient in the nutrient medium. Moreover, the work of several researchers revealed that natural abundance  $\delta^{15}\text{N}$  of plants reflects the net effect of a range of processes (Shibuya *et al.*, 2006). In this regard, the presence of multiple N-sources with distinct isotopic values, mycorrhizal associations, temporal and spatial variation in N availability, and changes in plant demand can all influence plant  $\delta^{15}\text{N}$ . In addition, the farm at higher altitude

may be planted with legume shade trees where appears and may supply N by atmospheric nitrogen fixation processes instead of discriminating against  $\delta^{15}\text{N}$ . Consequently, the  $\delta^{15}\text{N}$  values of leguminous plants are often close to 0‰ (Ehleringer and Rundel, 1989). Ehleringer and Rundel (1989) showed that roughly 30% of the nitrogen fixed by a legume was transferred to the associated coffee trees. Snoeck, *et al.* (2000) also proposed certain amount of N transferred by legume to sole coffee trees *via* litter fall.

The mean value for  $\delta^{13}\text{C}$  at 1150 meters above sea level was significantly lower than the mean value at 1545 and 1802 meters above sea level. There was a significant effect of shading on  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  levels of green coffee beans but not on %N and %C. For  $\delta^{13}\text{C}$  mean value at 1150 meters above sea level was significantly lower as compared to the value at 1545 and 1802 m a.s.l. and the shaded coffee showed higher mean value most probably due to better temperature regulation for

photosynthetic process of higher altitudes and shade. In  $\text{C}_3$  plants, discrimination against  $^{13}\text{C}$  by the carboxylating enzyme, Rubisco ( $\gg 27\text{‰}$ ), is linked to photosynthesis via the ratio of intercellular to atmospheric  $\text{CO}_2$  concentrations ( $\text{ci/ca}$ ) (Dawson *et al.* (2002)). This ratio reflects the relative magnitudes of net assimilation and stomatal conductance that relate to demand and supply of  $\text{CO}_2$ , respectively. Carbon-13 data are thus a useful index for assessing intrinsic water use efficiency ( $A/g$ ; the ratio of carbon acquired to water vapor losses via stomatal conductance,  $g$ ) and may even provide information on actual water use efficiency (the ratio of assimilation to transpiration) when the leaf-to-air vapor pressure difference is known (Ehleringer and Vogel, 1993). A few studies are now using C isotope data to investigate competition how the efficiency of resource use varied in the presence or absence of different neighbours (Williams *et al.* 1991). With regard to  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ , unshaded coffee showed higher mean value than the shaded counterpart.

Table 2. Effect of altitude, shade and processing Methods on Stable Isotopes and %N and %C

| Factor   | Level     | %N                   | $\delta^{15}\text{N}$ | %C                    | $\delta^{13}\text{C}$  | $\delta^{18}\text{O}$ |
|----------|-----------|----------------------|-----------------------|-----------------------|------------------------|-----------------------|
| Altitude | 1150 (12) | 2.4±0.1 <sup>a</sup> | 5.9±0.3 <sup>a</sup>  | 46.1±0.8 <sup>a</sup> | -27.6±0.2 <sup>b</sup> | 31.4±0.3 <sup>a</sup> |
|          | 1545(12)  | 2.4±0.1 <sup>a</sup> | 5.3±0.3 <sup>a</sup>  | 46.8±0.8 <sup>a</sup> | -27.6±0.2 <sup>b</sup> | 32.1±0.3 <sup>a</sup> |
|          | 1802(12)  | 2.4±0.1 <sup>a</sup> | 4.4±0.3 <sup>b</sup>  | 46.5±0.8 <sup>a</sup> | -26.7±0.2 <sup>a</sup> | 31.4±0.3 <sup>a</sup> |
|          | P-value   | 0.770                | 0.006                 | 0.861                 | <0.001                 | 0.184                 |
| Shade    | Shaded    | 2.4±0.0              | 5.0±0.3               | 46.3±0.7              | -27.6±0.1              | 31.1±0.2              |
|          | Unshaded  | 2.4±0.0              | 5.3±0.3               | 46.7±0.7              | -27.0±0.1              | 32.1±0.2              |
|          | P-value   | 0.904                | 0.419                 | 0.712                 | 0.005                  | 0.005                 |
| GM       |           | 2.4±0.0              | 5.2±0.2               | 46.5±0.5              | -27.3±0.1              | 31. ±0.2              |
| SD       |           | 0.2                  | 1.2                   | 2.5                   | 0.7                    | 1.7                   |
| CV%      |           | 6.5                  | 23.3                  | 5.4                   | -2.7                   | 5.4                   |

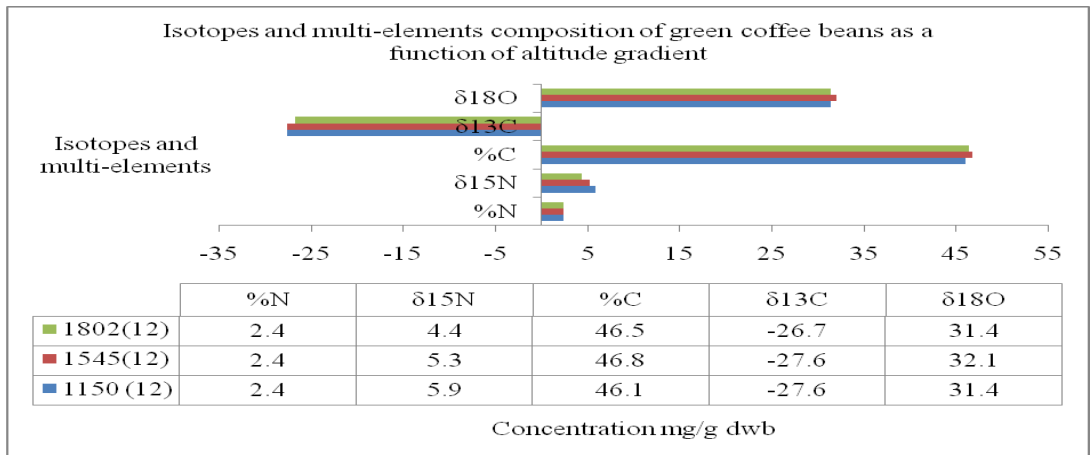


Figure 1. Isotopes and multi-element composition of green coffee beans as a function of altitude gradient

With regard to  $\delta^{18}\text{O}$  unshaded coffee showed higher mean value as compared to shaded counterpart likely due to better accumulation of the heavy isotope at unshaded condition because of less humidity as compared to humid shaded condition. Oxygen isotope ratios have been most useful in tracing and describing water movement and as indicators of humidity regimes. Oxygen derived from  $\text{CO}_2$  undergoes a complete exchange with the oxygen of the water in the plant during the synthesis of cellulose, and thus  $\delta^{18}\text{O}$  of tissue water is the primary influence on the  $\delta^{18}\text{O}$  of fixed oxygen in cellulose (Ehleringer and Rundel, 1989). The  $\delta^{18}\text{O}$  generally varies with ambient humidity, which in turn reflects changes in water use (Meinzer, *et al.*, 1990). The  $\delta^{18}\text{O}$  of leaf and tree ring cellulose are largely determined by the integrated leaf-to-air vapor pressure gradient during photosynthetic gas exchange (Ehleringer and Vogel, 1993). This leaf-air vapour pressure gradient changes with environmental

conditions (atmospheric humidity, soil moisture, air temperature) and plant response to these environmental changes (e.g., changes in water use, leaf temperature, and net assimilation). Measurement of the  $\delta^{18}\text{O}$  composition of plant tissues may thus aid for the interpretation of differences in  $\delta^{13}\text{C}$  among individual plants growing in the same location and among species in different environments.

The bi-factor interaction effect of Altitude \* Shade, and Altitude \* Processing methods were significant for  $\delta^{18}\text{O}$  (Table s 3 & 4), and non-significant, otherwise. Shading disfavoured the composition of  $\delta^{18}\text{O}$  in green coffee beans as the altitude increases while favouring at lower altitude. Unwashed coffee showed higher  $\delta^{18}\text{O}$  composition of green coffee beans at higher altitudes but lower at low altitude. Both shading and washing could discriminate coffees of higher altitudes from that of lowlands. The tri-factor interactions resulted in significant effect of none of

the response variables (Table 3). Moreover, the determination of water use efficiency (WUE) is greatly improved by the simultaneous use of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in plant tissues (Saurer *et al.* 1997). By considering concurrent variations  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , one can distinguish between biochemical and stomatal limitations to photosynthesis in response to a change in environmental conditions.

Shading disfavoured the composition of  $\delta^{18}\text{O}$  in green coffee beans as the altitude increases while favouring at lower altitude. This indicates shading requirement reduces as altitude increases because of lower amplitude of temperature and higher relative humidity. Bote and Struik (2011) noted that shade triggers differences in physiological behaviour of the coffee plants, such as improved photosynthesis and increased leaf area index, resulting in better performance than possible in direct sun light. Shaded plants had greater biochemical and physiological potential for high dry matter production which would help them to maintain high coffee yields in the long term. Processing methods did not show any significant effect on any of the studied variables. Unwashed coffee showed higher  $\delta^{18}\text{O}$  composition of green coffee beans at higher altitudes but lower at low altitude. Both shading and washing could discriminate coffees of higher altitudes from that of lowlands.

Rodrigues *et al.* (2009) observed differences on stable isotopic and elemental composition and associated

them by altitude and precipitation values associated with the different geographic locations. The isotopic compositions of carbon and nitrogen, which are the main elements of living organisms, are used as proxies for environmental parameters that characterize a certain crop or area (Meinzer *et al.*, 1990). Since different elements represent different characteristics of the environment (water cycle, plant physiology, agricultural practices, hydric stress, soil geology, etc.), the combination of these indicators may be used to unequivocally identify a certain ecosystem of origin. A noticeable feature of using stable isotope abundance data, in a multivariate approach to discriminate the origin of a product, is that each single isotopic signature is in fact the result of more than one unique phenomenon, and is thus very difficult to counterfeit for fraud purposes.

A study by Sera *et al.* (2005) showed that the small climatic differences among regions which do not have very marked differences may show little isotopic variation. Stable isotope ratios of carbon, nitrogen and boron have been shown to be good indicators of geographical-dependent parameters, and therefore to be useful tools to infer the region of production of green coffee (Rodrigues *et al.*, 2009; Adugnaw, 2014). An inherent limitation of isotope ratio techniques is due to the mismatching between national borders and climatic borders, delimiting different geographical regions; for this reason, coffees

produced in small adjacent countries with similar climatic conditions cannot be distinguished from one another on the basis of different isotope ratio values, while large countries with a

large variety of climatic areas may show samples with a range of isotope ratio values, displaying wide dispersion.

Table 3. Mean effect of bi-factor interactions on the composition of stable isotopes and %N & %C of green coffee beans

| Altitude * Shade |          | %N      | $\delta^{15}\text{N}$ | %C       | $\delta^{13}\text{C}$ | $\delta^{18}\text{O}$ |
|------------------|----------|---------|-----------------------|----------|-----------------------|-----------------------|
| 1150             | Shaded   | 2.3±0.1 | 5.4±0.4               | 45.9±1.2 | -27.9±0.2             | 32.2±0.4              |
|                  | Unshaded | 2.4±0.1 | 6.4±0.4               | 46.4±1.2 | -27.4±0.2             | 30.6±0.4              |
| 1545             | Shaded   | 2.4±0.1 | 5.4±0.4               | 46.5±1.2 | -27.8±0.2             | 31.6±0.4              |
|                  | Unshaded | 2.4±0.1 | 5.2±0.4               | 47.1±1.2 | -27.3±0.2             | 32.5±0.4              |
| 1802             | Shaded   | 2.4±0.1 | 4.3±0.4               | 46.5±1.2 | -27.1±0.2             | 29.4±0.4              |
|                  | Unshaded | 2.4±0.1 | 4.4±0.4               | 46.6±1.2 | -26.3±0.2             | 33.3±0.4              |
| P-value          |          | 0.89    | 0.442                 | 0.967    | 0.676                 | <0.001                |

Table 4. Mean effect of bi-factor interaction on the composition of stable isotopes and %N & %C of green coffee beans

| Altitude * Processing |          | %N      | $\delta^{15}\text{N}$ | %C       | $\delta^{13}\text{C}$ | $\delta^{18}\text{O}$ |
|-----------------------|----------|---------|-----------------------|----------|-----------------------|-----------------------|
| 1150                  | Washed   | 2.3±0.1 | 5.9±0.4               | 45.7±1.2 | -27.4±0.2             | 31.7±0.4              |
|                       | Unwashed | 2.4±0.1 | 6.0±0.4               | 46.5±1.2 | -27.9±0.2             | 31.0±0.4              |
| 1545                  | Washed   | 2.3±0.1 | 5.7±0.4               | 46.2±1.2 | -27.6±0.2             | 31.0±0.4              |
|                       | Unwashed | 2.4±0.1 | 4.9±0.4               | 47.4±1.2 | -27.5±0.2             | 33.1±0.4              |
| 1802                  | Washed   | 2.4±0.1 | 4.7±0.4               | 45.9±1.2 | -26.5±0.2             | 31.2±0.4              |
|                       | Unwashed | 2.4±0.1 | 4.0±0.4               | 47.2±1.2 | -26.8±0.2             | 31.5±0.4              |
| P-value               |          | 0.875   | 0.552                 | 0.977    | 0.365                 | 0.01                  |

## Biochemical composition of green coffee beans as a function of altitude, shade and processing methods

Altitude had significant effect ( $P \leq 0.05$ ) on 4-Caffeoylquinic acid (4-CQA), 5-Caffeoylquinic acid (5-CQA), Feruloylquinic acids (FQA), 3,4-Dicaffeoylquinic acids (3,4-DCQA), 4,5-Dicaffeoylquinic acids (4,5-DCQA), CFQA, Total Chlorogenic Acids (TCGA), sucrose, trigonelline composition of green coffee beans. However, altitude did not have a

significant effect ( $P > 0.05$ ) on Caffeine and 3-CQA compositions. For 4-CQA and 5-CQA lowland samples showed the least mean value as compared to both midland and highland samples. On the other hand, for FQA, 4,5-DCQA, and TCGA, the highland sample showed the least mean value as compared to both lowland and midland samples.

The compositions of 3,4-DCQA, CFQA, and sucrose were the lowest for samples of coffee beans obtained from the lowlands compared to those

obtained from both midland and highlands. The effect of shade was significant for Caffeoylferuloylquinic acids (CFQA), and non-significant, otherwise. Unshaded coffee gave higher mean value of CFQA composition of green coffee beans as compared to shaded counterpart. Processing methods had significant effect on 3-CQA, 5-CQA, 4,5-DCQA, CFQA, TCGA and trigonelline composition, and non-significant, otherwise. Washed coffee showed higher mean value for 5-CQA, 4,5-DCQA, TCGA, and trigonelline composition of green coffee beans while unwashed coffee resulted in higher mean value for 3-CQA and CFQA composition of green coffee beans. As could be seen on Figure 2 the mean value for 3,4-DCQA, 4,5-DCQA, CFQA, TCGA, sucrose, and trigonelline decreased in the order of lowland, midland and highland samples. However, for caffeine, 4-CQA, and 5-CQA the value increased in the order of midland, highland and lowland where as for 3-CQA and FQA in the order of midland, lowland and highland samples.

The effect of Altitude\*Shade interaction was significant for sucrose and trigonelline composition of green coffee beans, and non-significant, otherwise (Table 5). In this regard, shaded coffee at 1150 meters above sea level (m a.s.l.) showed the highest mean value while at 1802 m a.s.l. the lowest for both sucrose and trigonelline composition of green coffee beans. The effect of Altitude\*Processing was significant

for 5-CQA, CFQA, TCGA, sucrose and trigonelline of composition of green coffee beans (Table 6). Washed coffee at 1802 m a.s.l. for 5-CQA, unwashed coffee at 1150 m a.s.l. for CFQA, unwashed coffee at 1545 m a.s.l. for TCGA, unwashed coffee at 1150 m a.s.l. for both sucrose and trigonelline showed the highest mean value while unwashed coffee at 1802 m a.s.l. for 5-CQA, TCGA, sucrose and trigonelline, and washed coffee at 1545 m a.s.l. for CFQA showed the least value. The study did not observe any significant effect of Shade\* Processing methods. The Tri-Factor Interaction Effect of Altitude, Shade and Processing methods showed significant effect on 5-CQA composition of green coffee beans, and non-significant, otherwise (Table 7). In this regard, the highest mean value was obtained from unwashed coffee and shaded coffee at 1545 m a.s.l. while unwashed coffee and shaded coffee at 1802 was the lowest.

For 4-CQA and 5-CQA lowland samples showed the least mean value as compared to both midland and highland samples. The composition of 3,4-DCQA, CFQA, and sucrose was significantly the lowest at lowland compared to both midland and highland samples. On the other hand, for FQA, 4,5-DCQA, and TCGA, the highland sample showed the least mean value as compared to both lowland and midland samples. A study by Bertrand *et al.* (2006) is in complement with this result showing that elevation had a significant effect on bean biochemical composition,

with chlorogenic acid and fat concentrations increasing with increasing elevation for some varieties and little effect on the variation of

chlorogenic acid concentration but not on fat concentration.

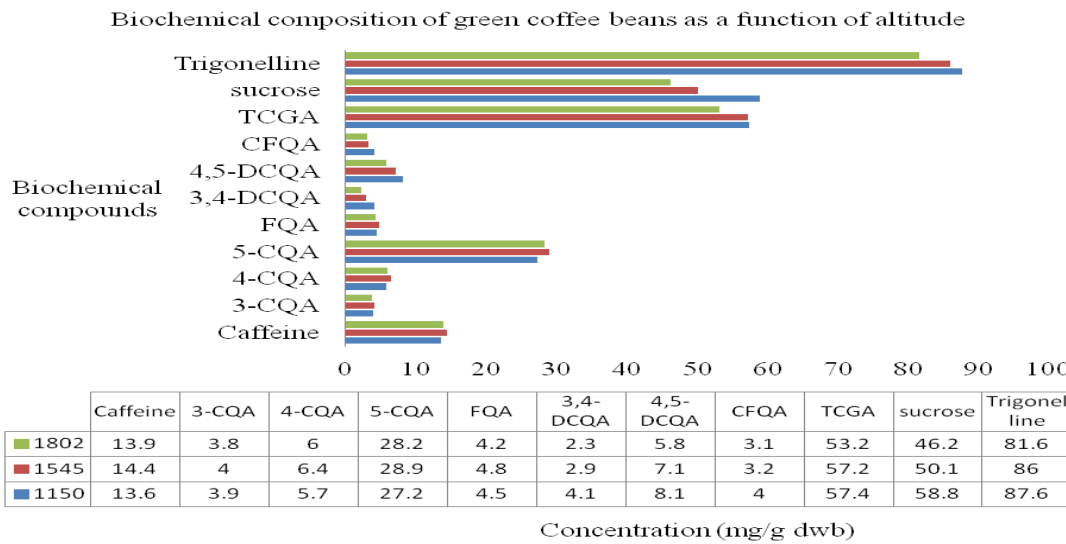


Figure 2. Biochemical composition of green coffee beans as a function of altitude gradient

Table 5. Effect of Altitude, shade and processing methods on biochemical composition of green coffee beans

|                           | Caffeine              | 3-CQA                | 4-CQA                | 5-CQA                 | FQA                  | 3,4-DCQA             | 4,5-DCQA             | CFQA                 | TCGA                  | sucrose               | trigonelline          |
|---------------------------|-----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|-----------------------|
| <b>Altitude</b>           |                       |                      |                      |                       |                      |                      |                      |                      |                       |                       |                       |
| 1150 (120)                | 13.6±0.2 <sup>a</sup> | 3.9±0.2 <sup>a</sup> | 5.7±0.1 <sup>b</sup> | 27.2±0.3 <sup>b</sup> | 4.5±0.2 <sup>a</sup> | 4.1±0.2 <sup>a</sup> | 8.1±0.3 <sup>a</sup> | 4.0±0.1 <sup>a</sup> | 57.4±0.6 <sup>a</sup> | 58.8±2.2 <sup>a</sup> | 87.6±0.3 <sup>a</sup> |
| 1545 (12)                 | 14.4±0.2 <sup>a</sup> | 4.0±0.2 <sup>a</sup> | 6.4±0.1 <sup>a</sup> | 28.9±0.3 <sup>a</sup> | 4.8±0.2 <sup>a</sup> | 2.9±0.2 <sup>b</sup> | 7.1±0.3 <sup>a</sup> | 3.2±0.1 <sup>b</sup> | 57.2±0.6 <sup>a</sup> | 50.1±2.2 <sup>b</sup> | 86.0±0.3 <sup>b</sup> |
| 1802 (12)                 | 13.9±0.2 <sup>a</sup> | 3.8±0.2 <sup>a</sup> | 6.0±0.1 <sup>a</sup> | 28.2±0.3 <sup>a</sup> | 4.2±0.2 <sup>b</sup> | 2.3±0.2 <sup>b</sup> | 5.8±0.3 <sup>b</sup> | 3.1±0.1 <sup>b</sup> | 53.2±0.6 <sup>b</sup> | 46.2±2.2 <sup>b</sup> | 81.6±0.3 <sup>c</sup> |
| P-value                   | 0.08                  | 0.585                | 0.013                | 0.002                 | 0.014                | <0.001               | <0.001               | <0.001               | <0.001                | 0.001                 | <0.001                |
| <b>Shade</b>              |                       |                      |                      |                       |                      |                      |                      |                      |                       |                       |                       |
| Shaded                    | 14.2±0.2              | 3.8±0.1              | 5.9±0.1              | 27.9±0.2              | 4.6±0.1              | 2.9±0.2              | 7.2±0.2              | 3.2±0.1              | 55.4±0.5              | 52.9±1.8              | 84.8±0.3              |
| Unshaded                  | 13.7±0.2              | 4.1±0.1              | 6.1±0.1              | 28.2±0.2              | 4.4±0.1              | 3.2±0.2              | 6.8±0.2              | 3.7±0.1              | 56.5±0.5              | 50.5±1.8              | 85.3±0.3              |
| P-value                   | 0.111                 | 0.123                | 0.201                | 0.531                 | 0.259                | 0.215                | 0.354                | 0.002                | 0.12                  | 0.344                 | 0.274                 |
| <b>Processing methods</b> |                       |                      |                      |                       |                      |                      |                      |                      |                       |                       |                       |
| Washed                    | 14.2±0.2              | 3.7±0.1              | 6.0±0.1              | 28.5±0.2              | 4.6±0.1              | 3.1±0.2              | 8.1±0.2              | 2.8±0.1              | 56.7±0.5              | 49.2±1.8              | 85.9±0.3              |
| Unwashed                  | 13.7±0.2              | 4.1±0.1              | 6.0±0.1              | 27.6±0.2              | 4.3±0.1              | 3.1±0.2              | 5.9±0.2              | 4.1±0.1              | 55.2±0.5              | 54.2±1.8              | 84.2±0.3              |
| P-value                   | 0.111                 | 0.029                | 0.867                | 0.025                 | 0.085                | 0.945                | <0.001               | <0.001               | 0.029                 | 0.064                 | <0.001                |

Table 6. The Bi-Factor Interaction Effect of Altitude, Shade and Processing methods on Biochemical Composition of Green Coffee Beans

|                            |                 | Caffeine | 3-CQA   | 4-CQA   | 5-CQA    | FQA     | 3,4-DCQA | 4,5-DCQA | CFQA    | TCGA     | sucrose  | trigonelline |
|----------------------------|-----------------|----------|---------|---------|----------|---------|----------|----------|---------|----------|----------|--------------|
| <b>Altitude*Shade</b>      |                 |          |         |         |          |         |          |          |         |          |          |              |
| 1150                       | Shaded          | 14.1±0.3 | 4.0±0.2 | 5.7±0.2 | 26.9±0.4 | 4.7±0.2 | 4.0±0.3  | 7.9±0.4  | 3.8±0.2 | 57.1±0.8 | 64.7±3.1 | 88.0±0.5     |
|                            | Unshaded        | 13.1±0.3 | 3.9±0.2 | 5.7±0.2 | 27.4±0.4 | 4.2±0.2 | 4.2±0.3  | 8.2±0.4  | 4.2±0.2 | 57.7±0.8 | 53.0±3.1 | 87.3±0.5     |
| 1545                       | Shaded          | 14.6±0.3 | 3.8±0.2 | 6.3±0.2 | 28.8±0.4 | 4.9±0.2 | 2.7±0.3  | 7.4±0.4  | 2.9±0.2 | 56.7±0.8 | 48.4±3.1 | 85.0±0.5     |
|                            | Unshaded        | 14.1±0.3 | 4.2±0.2 | 6.5±0.2 | 29.0±0.4 | 4.8±0.2 | 3.0±0.3  | 6.8±0.4  | 3.5±0.2 | 57.8±0.8 | 51.8±3.1 | 87.0±0.5     |
| 1802                       | Shaded          | 13.8±0.3 | 3.5±0.2 | 5.7±0.2 | 28.2±0.4 | 4.1±0.2 | 2.1±0.3  | 6.1±0.4  | 2.9±0.2 | 52.5±0.8 | 45.7±3.1 | 81.5±0.5     |
|                            | Unshaded        | 13.9±0.3 | 4.1±0.2 | 6.2±0.2 | 28.2±0.4 | 4.2±0.2 | 2.5±0.3  | 5.5±0.4  | 3.3±0.2 | 53.9±0.8 | 46.7±3.1 | 81.6±0.5     |
|                            | P-value         | 0.295    | 0.480   | 0.366   | 0.883    | 0.354   | 0.878    | 0.428    | 0.618   | 0.901    | 0.049    | 0.019        |
| 1150                       | Washed coffee   | 13.8±0.3 | 3.8±0.2 | 5.7±0.2 | 27.3±0.4 | 4.7±0.2 | 4.1±0.3  | 9.3±0.4  | 3.1±0.2 | 57.8±0.8 | 49.4±3.1 | 87.6±0.5     |
| <b>Altitude*Processing</b> |                 |          |         |         |          |         |          |          |         |          |          |              |
| 1545                       | Unwashed coffee | 13.4±0.3 | 4.1±0.2 | 5.8±0.2 | 27.0±0.4 | 4.2±0.2 | 4.1±0.3  | 6.8±0.4  | 5.0±0.2 | 57.0±0.8 | 68.2±3.1 | 87.7±0.5     |
|                            | Washed coffee   | 14.4±0.3 | 3.8±0.2 | 6.3±0.2 | 28.6±0.4 | 4.8±0.2 | 2.7±0.3  | 7.8±0.4  | 2.4±0.2 | 56.4±0.8 | 50.3±3.1 | 84.3±0.5     |
| 1802                       | Unwashed coffee | 14.3±0.3 | 4.3±0.2 | 6.4±0.2 | 29.1±0.4 | 4.9±0.2 | 3.0±0.3  | 6.5±0.4  | 4.0±0.2 | 58.1±0.8 | 49.9±3.1 | 87.6±0.5     |
|                            | Washed coffee   | 14.3±0.3 | 3.6±0.2 | 6.0±0.2 | 29.5±0.4 | 4.5±0.2 | 2.5±0.3  | 7.2±0.4  | 2.8±0.2 | 55.9±0.8 | 48.0±3.1 | 85.8±0.5     |
|                            | Unwashed coffee | 13.4±0.3 | 4.0±0.2 | 5.9±0.2 | 26.8±0.4 | 3.9±0.2 | 2.2±0.3  | 4.5±0.4  | 3.3±0.2 | 50.5±0.8 | 44.4±3.1 | 77.3±0.5     |
|                            | P-value         | 0.444    | 0.933   | 0.933   | 0.003    | 0.199   | 0.6      | 0.165    | 0.002   | 0.001    | 0.003    | <0.001       |

Table 7. The Tri-Factor Interaction Effect of Altitude, Shade and Processing on Biochemical Composition of Green Coffee Beans (mg/g dwb)

| Altitude | Shade    | Processing methods | Caffeine | 3-CQA   | 4-CQA   | 5-CQA    | FQA     | 3,4-DCQA | 4,5-DCQA | CFQA    | TCGA     | sucrose  | trigonelline |
|----------|----------|--------------------|----------|---------|---------|----------|---------|----------|----------|---------|----------|----------|--------------|
| 1150     | Shaded   | Washed coffee      | 14.2±0.5 | 3.8±0.3 | 5.7±0.3 | 26.6±0.6 | 5.0±0.3 | 4.1±0.4  | 9.2±0.6  | 3.0±0.2 | 57.2±1.1 | 50.8±4.4 | 87.8±0.7     |
|          |          | Unwashed coffee    | 13.9±0.5 | 4.1±0.3 | 5.8±0.3 | 27.2±0.6 | 4.5±0.3 | 3.9±0.4  | 6.6±0.6  | 4.7±0.2 | 56.9±1.1 | 78.5±4.4 | 88.2±0.7     |
|          | Unshaded | Washed coffee      | 13.3±0.5 | 3.7±0.3 | 5.6±0.3 | 27.9±0.6 | 4.4±0.3 | 4.1±0.4  | 9.4±0.6  | 3.2±0.2 | 58.3±1.1 | 48.0±4.4 | 87.3±0.7     |
|          | Shaded   | Unwashed coffee    | 12.9±0.5 | 4.1±0.3 | 5.7±0.3 | 26.8±0.6 | 4.0±0.3 | 4.2±0.4  | 7.0±0.6  | 5.2±0.2 | 57.1±1.1 | 57.9±4.4 | 87.2±0.7     |
| 1545     | Unshaded | Washed coffee      | 14.6±0.5 | 3.5±0.3 | 6.2±0.3 | 29.5±0.6 | 4.5±0.3 | 2.2±0.4  | 7.8±0.6  | 1.9±0.2 | 55.6±1.1 | 46.5±4.4 | 83.1±0.7     |
|          |          | Unwashed coffee    | 14.6±0.5 | 4.2±0.3 | 6.3±0.3 | 28.0±0.6 | 5.3±0.3 | 3.2±0.4  | 7.1±0.6  | 3.9±0.2 | 57.8±1.1 | 50.4±4.4 | 86.8±0.7     |
|          | Shaded   | Washed coffee      | 14.2±0.5 | 4.0±0.3 | 6.4±0.3 | 27.7±0.6 | 5.0±0.3 | 3.2±0.4  | 7.7±0.6  | 3.0±0.2 | 57.2±1.1 | 54.1±4.4 | 85.5±0.7     |
|          |          | Unwashed coffee    | 14.0±0.5 | 4.4±0.3 | 6.5±0.3 | 30.2±0.6 | 4.5±0.3 | 2.9±0.4  | 5.9±0.6  | 4.1±0.2 | 58.3±1.1 | 49.4±4.4 | 88.5±0.7     |
| 1802     | Unshaded | Washed coffee      | 14.5±0.5 | 3.2±0.3 | 5.6±0.3 | 29.8±0.6 | 4.4±0.3 | 2.1±0.4  | 7.7±0.6  | 2.4±0.2 | 55.2±1.1 | 48.0±4.4 | 85.9±0.7     |
|          | Shaded   | Unwashed coffee    | 13.1±0.5 | 3.9±0.3 | 5.8±0.3 | 26.5±0.6 | 3.9±0.3 | 2.1±0.4  | 4.5±0.6  | 3.3±0.2 | 49.9±1.1 | 43.3±4.4 | 77.2±0.7     |
|          | Unshaded | Washed coffee      | 14.2±0.5 | 4.0±0.3 | 6.4±0.3 | 29.1±0.6 | 4.6±0.3 | 2.8±0.4  | 6.7±0.6  | 3.2±0.2 | 56.7±1.1 | 48.1±4.4 | 85.8±0.7     |
|          |          | Unwashed coffee    | 13.7±0.5 | 4.1±0.3 | 6.1±0.3 | 27.2±0.6 | 3.8±0.3 | 2.3±0.4  | 4.4±0.6  | 3.3±0.2 | 51.2±1.1 | 45.4±4.4 | 77.4±0.7     |
| P-value  |          |                    | 0.714    | 0.721   | 0.799   | 0.007    | 0.299   | 0.41     | 0.397    | 0.108   | 0.96     | 0.293    | 0.881        |

## Stepwise Regression between isotopes and biochemical composition of green coffee beans

A stepwise multiple regressions were conducted to evaluate whether stable isotopes and multi-elements were necessary to predict biochemical composition of green coffee beans. The regression analysis between stable isotopes ( $\delta^{18}\text{O}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{13}\text{C}$ ), %N and %C, and biochemical compounds of green coffee beans showed significant contribution of the model for caffeine, 4,5-DCQA, TCGA, and sucrose composition of green coffee beans (Table 8) indicating significant positive regression weights. All the requested variables entered into the regression equation for all dependant variables at step 1 of the analysis. Thus, only one Model was employed. The model was significantly related to dependant variable  $F(5, 30) = 3.147$ ,  $p = 0.021$  for caffeine;  $= 5.007$ ,  $p = 0.002$  for 4,5-DCQA;  $= 3.292$ ,  $p = 0.017$  for

TCGA;  $= 2.672$ ,  $p = 0.041$  for sucrose (Table 9&10), and non-significant, otherwise. For caffeine, 4,5-DCQA, TCGA and sucrose about 34%, 46% 35% and 31% of the variation was accounted for by the model, respectively. For caffeine percent N contributed significant positive weight ( $b = 6.604$ ,  $p = 0.002$ ) and %C contributed significant negative ( $b = -0.388$ ,  $p = 0.004$ ), respectively) for the model. The study showed that all the studied stable isotopes did not contribute significant weight to the model. Moreover, a t-test showed that biochemical compounds in the list 3-CQA, 4-CQA, 5-CQA, FQA, and CFQA were not significantly influenced ( $p > 0.05$ ) by any of the predictors (Table 9&10). The study investigated that there was significant contribution of  $\delta^{15}\text{N}$  for variation of 4,5-DCQA ( $P = 0.002$ ), 3,4-DCQA ( $P = 0.043$ ), and TCGA ( $P = 0.003$ ).

Table 8. The Model for regressing biochemical compounds on stable isotopes and %N & %C content of coffee beans

| Dependant variable | Model | R     | R <sup>2</sup> | Adjusted R <sup>2</sup> | SE. of the Estimate | Model P-value (Regression) |
|--------------------|-------|-------|----------------|-------------------------|---------------------|----------------------------|
| Caffeine           | 1     | 0.587 | 0.344          | 0.235                   | 0.7926              | 0.021                      |
| trigonelline       | 1     | 0.474 | 0.225          | 0.096                   | 3.7218              | 0.155                      |
| Sucrose            | 1     | 0.555 | 0.308          | 0.193                   | 9.8744              | 0.041                      |
| 3-CQA              | 1     | 0.467 | 0.218          | 0.088                   | 0.5349              | 0.171                      |
| 3-CQA              | 1     | 0.467 | 0.218          | 0.088                   | 0.5349              | 0.171                      |
| 4-CQA              | 1     | 0.361 | 0.130          | -0.015                  | 0.5272              | 0.494                      |
| 5-CQA              | 1     | 0.305 | 0.093          | -0.058                  | 1.5686              | 0.689                      |
| FQA                | 1     | 0.454 | 0.206          | 0.0740                  | 0.5917              | 0.202                      |
| 4,5-DCQA           | 1     | 0.674 | 0.455          | 0.364                   | 0.7975              | 0.002                      |
| 3,4-DCQA           | 1     | 0.448 | 0.201          | 0.067                   | 1.6497              | 0.218                      |
| CFQA               | 1     | 0.428 | 0.183          | 0.047                   | 0.9391              | 0.272                      |
| TCGA               | 1     | 0.595 | 0.354          | 0.247                   | 2.6674              | 0.017                      |

Table 9. Stepwise Regression of caffeine, trigonelline, sucrose with stable isotopes and %N &amp; %C content of coffee beans

| Dependant variable | Independent variables in the model | Unstandardized Coefficients B | Std. Error | Standardized Coefficients Beta | t      | Sig.  |
|--------------------|------------------------------------|-------------------------------|------------|--------------------------------|--------|-------|
| Caffeine           | (Constant)                         | 4.272                         | 7.336      |                                | 0.582  | 0.565 |
|                    | %N                                 | 6.604                         | 1.958      | 1.134                          | 3.373  | 0.002 |
|                    | $\delta^{15}\text{N}$              | -0.044                        | 0.124      | -0.058                         | -0.352 | 0.728 |
|                    | %C                                 | -0.388                        | 0.122      | -1.071                         | -3.166 | 0.004 |
|                    | $\delta^{13}\text{C}$              | -0.280                        | 0.204      | -0.231                         | -1.373 | 0.180 |
|                    | $\delta^{18}\text{O}$              | 0.144                         | 0.083      | 0.270                          | 1.736  | 0.093 |
| trigonelline       | (Constant)                         | 43.020                        | 34.445     |                                | 1.249  | 0.221 |
|                    | %N                                 | -6.669                        | 9.194      | -0.265                         | -0.725 | 0.474 |
|                    | $\delta^{15}\text{N}$              | 1.247                         | 0.583      | 0.386                          | 2.139  | 0.041 |
|                    | %C                                 | 0.278                         | 0.575      | .177                           | 0.483  | 0.633 |
|                    | $\delta^{13}\text{C}$              | -0.975                        | 0.958      | -0.186                         | -1.018 | 0.317 |
|                    | $\delta^{18}\text{O}$              | 0.378                         | 0.389      | 0.165                          | .972   | 0.339 |
| Sucrose            | (Constant)                         | -252.614                      | 91.388     |                                | -2.764 | 0.010 |
|                    | %N                                 | -9.396                        | 24.394     | -0.133                         | -0.385 | 0.703 |
|                    | $\delta^{15}\text{N}$              | 0.840                         | 1.547      | 0.093                          | 0.543  | 0.591 |
|                    | %C                                 | 1.576                         | 1.526      | 0.359                          | 1.033  | 0.310 |
|                    | $\delta^{13}\text{C}$              | -7.836                        | 2.541      | -0.533                         | -3.084 | 0.004 |
|                    | $\delta^{18}\text{O}$              | 1.117                         | 1.032      | 0.173                          | 1.082  | 0.288 |

The stepwise Regression analysis showed significant positive influence of percent N and significant negative effect of percent C on green coffee bean caffeine composition but all the studied stable isotopes did not contribute significant weight to the model. Therefore, the weak association of stable isotopes and biochemical composition may require further investigation to be used as a tool for discriminating green coffee origins. The first function of the Canonical Discriminant Function completely separated coffees grown at 1150 m a.s.l. from those grown both at 1545 and 1802 m a.s.l. An experiment conducted in Central America by Bertrand *et al.* (2006) gave complementary result which discriminated the majority of the samples grown at high elevations from

the samples grown at the lowest elevations.

Most of the time for Arabica coffee (*Coffea arabica* L.) elevation and variety are important indicators of quality in world market. In conclusion, higher  $\delta^{15}\text{N}$  and higher  $\delta^{13}\text{C}$  values may be useful indicators of lowland and highland coffee samples, respectively.  $\delta^{15}\text{N}$  was found to be a significant indicator of variation of 4,5-DCQA; 3,4-DCQA ; and TCGA. Similarly, higher  $\delta^{18}\text{O}$  could discriminate highland unshaded coffees. The association of isotopes and biochemical composition of green coffee beans was found to be weak. In fact, for caffeine percent N contributed significant positive weight and %C significant negative weight, respectively for the model. This

implies the study revealed independency of the regressed variables each other. With the exception of caffeine  $\delta^{18}\text{O}$ , 3-CQA, %N, and %C most variables were useful predictors of altitudinal ranges of coffee growing sites. In fact, 4,5-

DCQA accounted for 94.4% of the variation composition of green coffee beans to classify lowland coffees from those at midland and highland grown coffees.

Table 10. Stepwise Regression of Chlorogenic acid families and stable isotopes and %N & %C content of coffee beans

| Dependant variable | Variables in the model | Unstandardized Coefficients B | Std. Error | Standardized Coefficients Beta | t      | Sig.  |
|--------------------|------------------------|-------------------------------|------------|--------------------------------|--------|-------|
| 3-CQA              | %N                     | -1.007                        | 1.321      | -.280                          | -.762  | 0.452 |
|                    | $\delta^{15}\text{N}$  | .136                          | .084       | .294                           | 1.621  | 0.116 |
|                    | %C                     | .113                          | .083       | .503                           | 1.364  | 0.183 |
|                    | $\delta^{13}\text{C}$  | -.063                         | .138       | -.084                          | -.456  | 0.652 |
|                    | $\delta^{18}\text{O}$  | .091                          | .056       | .278                           | 1.636  | 0.112 |
| 4-CQA              | %N                     | -.785                         | 1.302      | -.233                          | -.602  | 0.551 |
|                    | $\delta^{15}\text{N}$  | .105                          | .083       | .244                           | 1.274  | 0.212 |
|                    | %C                     | .066                          | .081       | .317                           | .813   | 0.422 |
|                    | $\delta^{13}\text{C}$  | .045                          | .136       | .064                           | .329   | 0.745 |
|                    | $\delta^{18}\text{O}$  | .077                          | .055       | .250                           | 1.394  | 0.173 |
| 5-CQA              | %N                     | -1.457                        | 3.875      | -.149                          | -.376  | 0.710 |
|                    | $\delta^{15}\text{N}$  | -.079                         | .246       | -.063                          | -.320  | 0.751 |
|                    | %C                     | -.029                         | .242       | -.048                          | -.121  | 0.905 |
|                    | $\delta^{13}\text{C}$  | .463                          | .404       | .227                           | 1.147  | 0.260 |
|                    | $\delta^{18}\text{O}$  | .005                          | .164       | .005                           | .028   | 0.978 |
| FQA                | %N                     | .810                          | 1.462      | .205                           | .554   | 0.584 |
|                    | $\delta^{15}\text{N}$  | .079                          | .093       | .156                           | .851   | 0.401 |
|                    | %C                     | -.032                         | .091       | -.132                          | -.355  | 0.725 |
|                    | $\delta^{13}\text{C}$  | -.267                         | .152       | -.325                          | -1.757 | 0.089 |
|                    | $\delta^{18}\text{O}$  | .109                          | .062       | .302                           | 1.761  | 0.088 |
| 4,5-DCQA           | %N                     | -1.833                        | 1.970      | -.285                          | -.931  | 0.359 |
|                    | $\delta^{15}\text{N}$  | .420                          | .125       | .509                           | 3.358  | 0.002 |
|                    | %C                     | .211                          | .123       | .529                           | 1.715  | 0.097 |
|                    | $\delta^{13}\text{C}$  | -.418                         | .205       | -.312                          | -2.037 | 0.051 |
|                    | $\delta^{18}\text{O}$  | .075                          | .083       | .127                           | .895   | 0.378 |
| 3,4-DCQA           | %N                     | -.434                         | 4.075      | -.040                          | -.106  | 0.916 |
|                    | $\delta^{15}\text{N}$  | .547                          | .259       | .388                           | 2.114  | 0.043 |
|                    | %C                     | -.084                         | .255       | -.122                          | -.328  | 0.745 |
|                    | $\delta^{13}\text{C}$  | -.095                         | .424       | -.041                          | -.223  | 0.825 |
|                    | $\delta^{18}\text{O}$  | -.033                         | .172       | -.033                          | -.191  | 0.850 |
| CFQA               | %N                     | 1.294                         | 2.320      | .209                           | .558   | 0.581 |
|                    | $\delta^{15}\text{N}$  | .104                          | .147       | .131                           | .705   | 0.486 |
|                    | %C                     | .019                          | .145       | .050                           | .133   | 0.895 |
|                    | $\delta^{13}\text{C}$  | -.365                         | .242       | -.283                          | -1.510 | 0.141 |
|                    | $\delta^{18}\text{O}$  | .124                          | .098       | .220                           | 1.265  | 0.216 |

## Canonical Discriminant Functions

A stepwise canonical discriminant function analysis of green coffee bean biochemical and stable isotopes and their respective element composition showed that 4,5-DCQA, TCGA, trigonelline,  $\delta^{13}\text{C}$ , 3,4-DCQA,  $\delta^{15}\text{N}$ , 4-CQA, sucrose, 5-CQA, FQA, CFQA in descending order of importance significantly ( $P \leq 0.05$ ) contributed for discrimination of altitudinal ranges (Table 11). At each step, the variable that minimizes the overall Wilks' Lambda was entered. About 83.3% of original grouped cases correctly classified. Two out of four functions were sufficient to accommodate the

variation up to 100%. In fact, the first function accommodated 94.4% of the variation which is contributed by 4,5-DCQA composition of green coffee beans (Figure 3). The first function completely separated coffees grown at 1150 m a.s.l. from those grown both at 1545 and 1802 m a.s.l. About 5.6% of the variation was accounted for by function 2 which separated 75% each of coffees grown at 1802 and 1545 m a.s.l. on one hand and about 70% of coffees grown at 1150 from that of 1802 m a.s.l., on the other. Both 4,5-DCQA and 4-CQA composition of green coffee beans contributed for by the variations at function 2.

Table 11. Tests of Equality of Group Means

| Predictor             | Wilks' Lambda | F      | df1 | df2 | Sig.  |
|-----------------------|---------------|--------|-----|-----|-------|
| 4,5-DCQA              | 0.442         | 20.824 | 2   | 33  | 0.000 |
| TCGA                  | 0.596         | 11.18  | 2   | 33  | 0.000 |
| trigonelline          | 0.558         | 13.069 | 2   | 33  | 0.000 |
| $\delta^{13}\text{C}$ | 0.637         | 9.415  | 2   | 33  | 0.001 |
| 3,4-DCQA              | 0.707         | 6.85   | 2   | 33  | 0.003 |
| $\delta^{15}\text{N}$ | 0.717         | 6.506  | 2   | 33  | 0.004 |
| 4-CQA                 | 0.732         | 6.043  | 2   | 33  | 0.006 |
| Sucrose               | 0.763         | 5.117  | 2   | 33  | 0.012 |
| 5-CQA                 | 0.784         | 4.533  | 2   | 33  | 0.018 |
| FQA                   | 0.793         | 4.32   | 2   | 33  | 0.022 |
| CFQA                  | 0.8           | 4.137  | 2   | 33  | 0.025 |
| Caffeine              | 0.861         | 2.674  | 2   | 33  | 0.084 |
| $\delta^{18}\text{O}$ | 0.962         | 0.654  | 2   | 33  | 0.526 |
| 3-CQA                 | 0.969         | 0.52   | 2   | 33  | 0.599 |
| %N                    | 0.98          | 0.332  | 2   | 33  | 0.72  |
| %C                    | 0.988         | 0.195  | 2   | 33  | 0.824 |

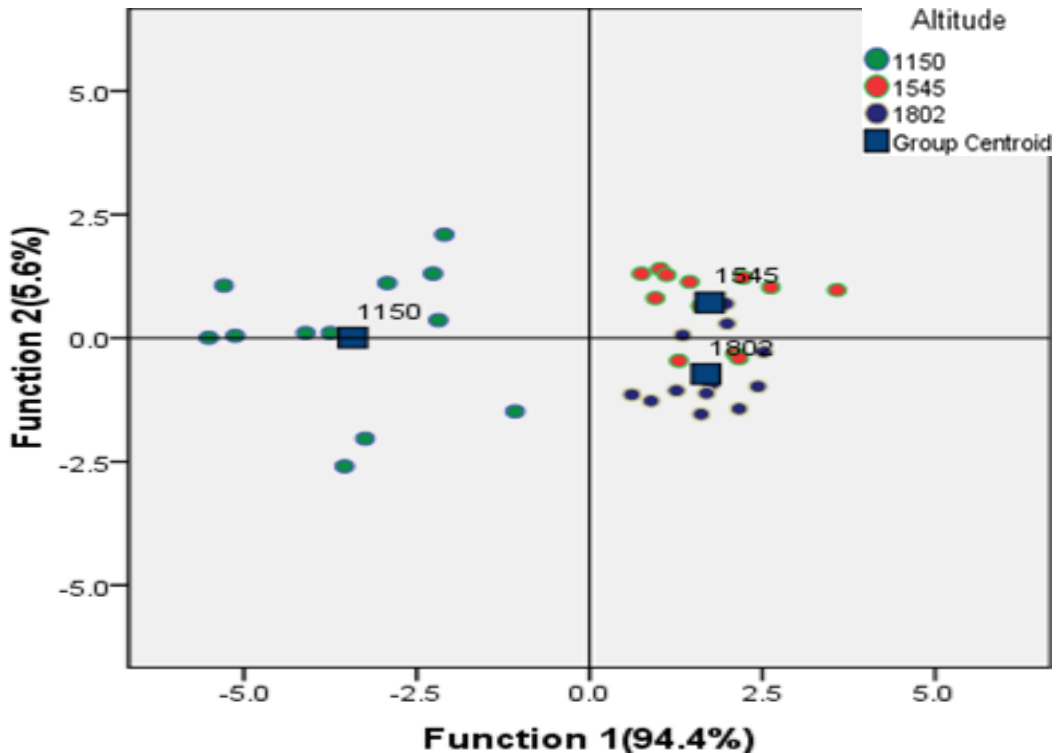


Figure 3 Canonical Discriminant Functions

Table 12. Eigenvalue and Wilks' Lambda

| Function | Eigenvalue         | % of Variance | Cumulative % | Canonical Correlation | Wilks' Lambda | Chi-square | df | Sig. |
|----------|--------------------|---------------|--------------|-----------------------|---------------|------------|----|------|
| 1        | 6.410 <sup>a</sup> | 94.4          | 94.4         | .930                  | .098          | 73.227     | 8  | .000 |
| 2        | .380 <sup>a</sup>  | 5.6           | 100.0        | .525                  | .725          | 10.138     | 3  | .017 |

a. First 2 canonical discriminant functions were used in the analysis

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# Value Chain Analysis of Wheat (*Triticum aestivum*): The Case of Dembecha District, West Gojjam Zone, Ethiopia

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## Abstract

*This study aimed to analyze the value chain of wheat in Dembecha district of West Gojjam zone. A total of 130 farm households, 30 traders, 2 bakers, 1 processor, 4 cooperatives and 10 consumers were used to collect the data. Functional and institutional approach of value chain analysis showed input providers, producers, cooperatives, collectors, wholesalers, retailers, and processors were found to be important wheat value chain actors. Four firms concentration ratio (CR4) was found to be 39.78% and there were information asymmetry and observed barriers to enter into wheat market. Buying, selling and pricing strategies showed deviation of wheat market from competitive market norms. Analysis of marketing margins revealed that processors received the highest (39.72%) marketing margin and retailers received the least marketing margins (4.85%) from consumers' price. Even if wheat in the study area deviate from competitive market norms as a result of oligopolistic market structure and conduct, profitability analysis of wheat market showed all market actors operated at profitable level. Supporting farmers and increase productivity through practical research, develop pie-growing mechanisms among actors, founding agro-processing firm, providing infrastructural facilities, designing advance way of disseminating market information, determining appropriate pricing strategies should be done to strength value chain development.*

**Keywords:** Dembecha, Market Concentration ratio, Pie-growing, Value chain, Wheat

## Introduction

Ethiopia's agricultural productions play a significant role in the local economy as a means of earning livelihoods for smallholder farmers, creating jobs and generating foreign exchange revenues (ATA, 2015; Wolfgang and Muluken, 2016).

However, the sector faces many challenges, making it more and more difficult to achieve its objective-feeding the nation each year. Population growth, urbanization, change in consumption habit, climate change, and other agricultural chain related constraints found at different stage of agricultural products chain

limit the significant share of the sector for most developing countries including Ethiopia (Acham and Rebecca, 2013).

Wheat (*Triticum aestivum*.) is one of the strategic cereal crops for the majority of Ethiopian's population. It plays an important role in the development of the agricultural sector and improvement in the income levels and livelihood situations of the farmers in developing countries. The contribution of wheat crop to national income was so large about 64% growth in total volume production in less than a decade (2006-2013) was achieved because of the wheat production sector (CSA, 2014). Moreover, the crop is high in terms of its contribution to food security and to the agricultural economy. It is the fourth importance crop in term of total gross value production and the second importance food next to maize in Ethiopia (FAO, 2015; ATA, 2015). With regard to the area of cultivation, wheat is the fourth most widely grown crop after *teff*, maize, and sorghum (CSA, 2017).

Wheat production and its marketing in Ethiopia is increasingly becoming as the means of livelihood for a million of smallholder households in Ethiopia and has grown significantly in the country in the past decade; represent an average annual growth of 7.5%. With respect to marketing, wheat production is the largest sub-sector in the economy and nearly 20% of the productions are marketed annually, which makes it second next to *teff*.

However, the production level of wheat and its value chain in Ethiopia are focused on the domestic level. Due to this reason wheat production level is not adequate enough and local consumption of wheat exceeds production and the country imports wheat for domestic demand. Between 25 and 35 percent of wheat is imported per annum for domestic consumption. Moreover, wheat manufacturing sub-sectors in the country performs below their capacity due wheat supply problem at the national level (Addis Ababa chamber of commerce and Sectoral Association) ACCSSA, (2017).

Smallholder farmers are the dominantly wheat producer, and accounts more 92% of the country wheat land. These farmers are characterized as subsistence oriented, low productivity, entirely dependent on weather with little investment in irrigation and characterized by the traditional market system (USAID, 2016). Regardless of the many promises held via decision makers to support these smallholders to solve their farming constraint, they still talk of dissatisfaction to the extent of support delivered to their pressing issues including; lack of adequate improved seed supply from seed enterprises, insufficient credit facilities, delayed pesticide delivery, lack of sufficient farm mechanization service provider, impracticality of research outcomes to growers first hand challenges.

Value chain analysis has attracted

considerable attention in recent years as a means to increase stabilize farm income and increase production, productivity and marketing agricultural product including wheat. It is also considered as a key strategy to improve market structure, and to reduce exposure to volatility of product supply and help to set coordinated linkages during product movement (Kirimi *et al.*, 2011; ILO, 2012). It helps to improve a given chain through coordinating value chain participants and a helpful instrument for serving the needs of a particular market sector. This helps to create buyer-driven value chains which characterized by knowledge intensity and market pull, relatively higher levels of technology or skills, customer order, or capital-intensive production practices (Efa *et al.*, 2016).

Lack of relevant market information, poor marketing alternatives, limited marketing institutions, and limited/absence of vertical or horizontal integration, absence/limited trust among value chain actors, inadequate or nonexistence coordination among chain actors and lack of marketing infrastructure such as storage, transportation services limits wheat value chain development (Yami *et al.*, 2012). Therefore, well-performing value chain and marketing system which satisfies consumer demands with the minimum margin between producers and consumer prices is essential to become competitive in this days business (Sultan, 2016). The existing bottlenecks/constraints had not yet

been studied and documented. This study, therefore, attempted to investigate the value chain of wheat, with a view to filling the existing gap, in Dembecha district of North Western Ethiopia specifically on identifying wheat value chain actors and their roles and also to analyze the structure, conduct and performance of wheat market in the study area.

## Research Methodology

### Description of the Study Area

Particularly the study was undertaken in Dembecha district. The district is one of the 15 districts of the western Gojjam zone in Amhara National Regional State. Dembecha is the name of the district as well as administrative center of the district and it is located at 205 km away from Bahir Dar town, Amhara regional state capital in Western direction on the main road from Bahir Dar to Addis Ababa. Dembecha is bordered in west by Bure, in the northwest by Jabi Tehnan, in the north by Dega Damot, and on the east and south by Misraq Gojjam. Towns in Dembecha district include Addis Alem, Dembecha, Zeleka and Yechereka. The district has 29 *kebeles* of which, 25 are rural while 4 are urban *kebeles* (Dembech District Livelihood report, 2017).

Agricultural production is the main means of livelihoods in the district. The main crops produced in the area include maize, wheat, sorghum, *teff* and pepper. Livestock husbandry is

dominated by cattle, sheep, goats, horse and donkey. The altitude of the district ranges from 1500 to 2999 meter above seas level. The agro ecological condition of Dembecha district is suitable for the production of cereals and grain legumes. About 87% of land area in

the district lies in mid-highland (*Woinadega*) whereas the remaining 12 % is high land (*dega*) and less than 1% is low land (*kolla*). The districts have monomodal rainfall distribution with average annual rainfall reaching 1006 mm (Dembech District Livelihood report, 2017).

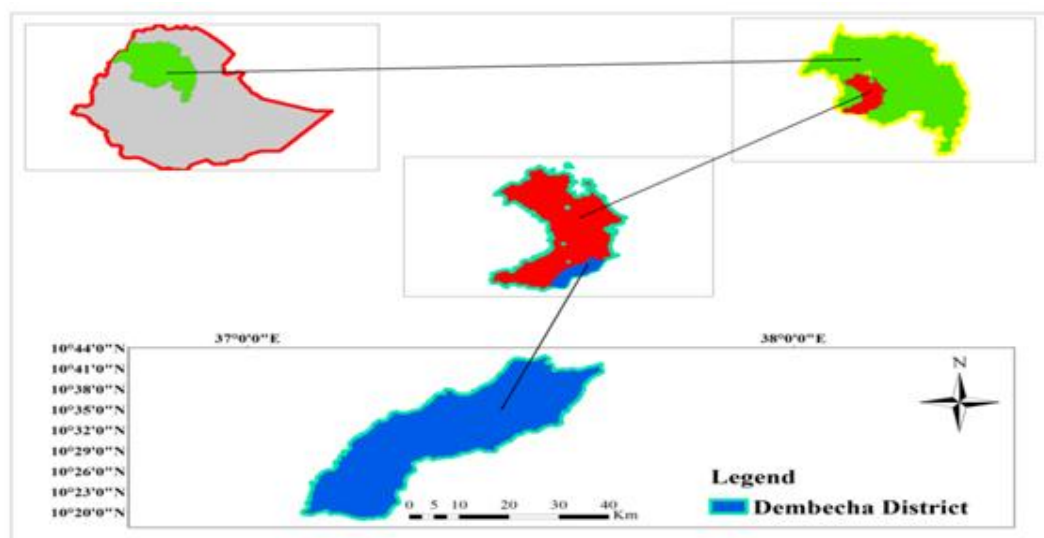


Figure 1. Location Map of Dembecha district

## Sampling method and sample size

Two stage-sampling techniques were employed to select sample wheat producer households. In the first stage, out of twenty-five wheat producers 'rural *kebeles* in Dembecha district, four *kebeles* namely Zeleka, Egziabherab, Jajjirab and Wad were selected randomly. Then, in the second stage wheat producers from the sample *kebeles* with the intended sample size were selected randomly using probability proportion to population

size sampling technique.

There is no common consensus on formula or rule of thumb that yields optimal sample size and the controversy is still unsettled. So, scholars have failed to reach common consensus, which leads various researchers to use various methods to determine sample size. Thus, based on the above justifications, a sample size of 130 sample households was drawn from the total of 4527 wheat producing households in the survey and therefore a sample size of 130 in

this case is adequate enough for generalizing findings. Hence, value chain research needs additional sample from chain participants a total of 30 traders, a census of 2 bakeries, a census of 1 processor and 4 cooperatives and also 10 consumers that purchase wheat from farmers in 2016/17 were used for the study.

## **Data sources and collection methods**

The study used primary and secondary data source to collect data. The primary data were collected by using of pre-tested semi structured interview schedule from selected farmers, traders, processor, bakers and consumers. The primary data collected from farmers include demographic and socio-economic characteristics, cost of production, quantities produced and sold, potential buyer of their product, and price related information, quantity sold to different alternatives, main input source experience in wheat farming and quantity of improved seed varieties used in 2016/17 production year.

Data from traders includes socio-demographic characteristics, type of trade, experience in wheat trade business, cost of transport used, selling and buying price of wheat, potential customers, accessibility of storage facilities, quantities purchased and sold, initial and current working capital, the amount of credit, purchasing strategy, sources and destinations of wheat and number of collection points. Secondary data

required for the study were taken from the Central Statistical Agency, published and unpublished sources, district agricultural and natural resource office, and trade and industry office of the Districts. To triangulate the answers provided by sample respondents, key informant interviews and focus group discussions were held with farmers, development agent and traders by using checklists.

## **Data Analysis Functional and Institutional Approach of Value Chain Analysis**

Value chain analyses (VCA) provide an overview of the actors, their activities, and flows of commodities, money and information and can identify challenges and suggest interventions (Kaplinsky and Morris, 2001). Functional and institutional approach of value chain analysis was used to study value chain actor, their roles and linkages. The following steps of value chain analysis were applied in this study. In first stage, the main value chain actors and their roles were identified and mapped. Then, based on the direction and volume of product flow the existing market channels and its performance were analyzed.

## **Structure-Conduct- Performance of Approach of market Analysis**

For this study, concentration ratio, market transparency (information) and entry barrier were used to measure the structure of the wheat market over Gini coefficient and Herfindahl Index

(HHI) because of both Gini Coefficient and Herfindahl Index require more data and it is difficult to achieve such full data in developing country like Ethiopia (Kotler, 2009).

$$CR_4 = \sum_{i=1}^4 S_i \quad i=1, 2, 3, 4$$

Where  $S_i$  = the percentage market share of  $i^{\text{th}}$  firm and  $i$  = four largest traders for which the ratio is going to be calculated.

In addition, the numbers of buyer and sellers (one or many), price setting strategy (trader, market and negotiation), quality determiner (standardize), bargaining power and the impact of physical location of the market on prices and marketing arrangements were taken for evaluating conduct of wheat market. Moreover, this study used marketing cost, margin and market return (profit) as a measure of wheat market performance across different channel.

Estimates of the marketing margins are the best tools to analyze performance of market and marketing margins of market actors were calculated by taking the difference between producers and retail prices. The producers share is the commonly employed ratio calculated mathematically as, the ratio of producers' price to consumers' price. Mathematically, producers 'share can obtain as follows:

$$PS = \frac{PP}{CP} \quad \text{and} \quad PS = 1 - \frac{MM}{CP}$$

Where: PS = Producer's share, PP = Producer's price, CP = Consumer

price and MM = marketing margin

$$TGMM = \frac{CP - PP}{CP} * 100$$

Where, TGMM = gross marketing margin, CP = consumer price, PP = producers price.

$$GMMT = \frac{CP - TGMM}{CP} * 100$$

Where, GMMT = marketing margin of traders, Sp = Selling price, Bp = Buying price

## Result and Discussion

### Map of wheat value chain

Value chain mapping enables to visualize the flow of the product from conception to end consumer through various actors. In this study, value chain analysis helps to identify the different actors involved in the wheat value chain, and to understand their roles and linkages. Consequently, the current value chain map of wheat is depicted in Figure 2. Based on this function, potential value chain actors were identified; their roles, functions, value adding processes, marketing and relationship were sorted out.

As depicted below, the map lists functions vertically along the left-hand side with the final markets across the top. The participants or actors of the value chain are designated by boxes at the middle. The enablers /supporters of wheat value chain are at the right-hand side of the chain. Product, information and financial flows are represented by single solid, double solid and single broken line arrows, respectively.

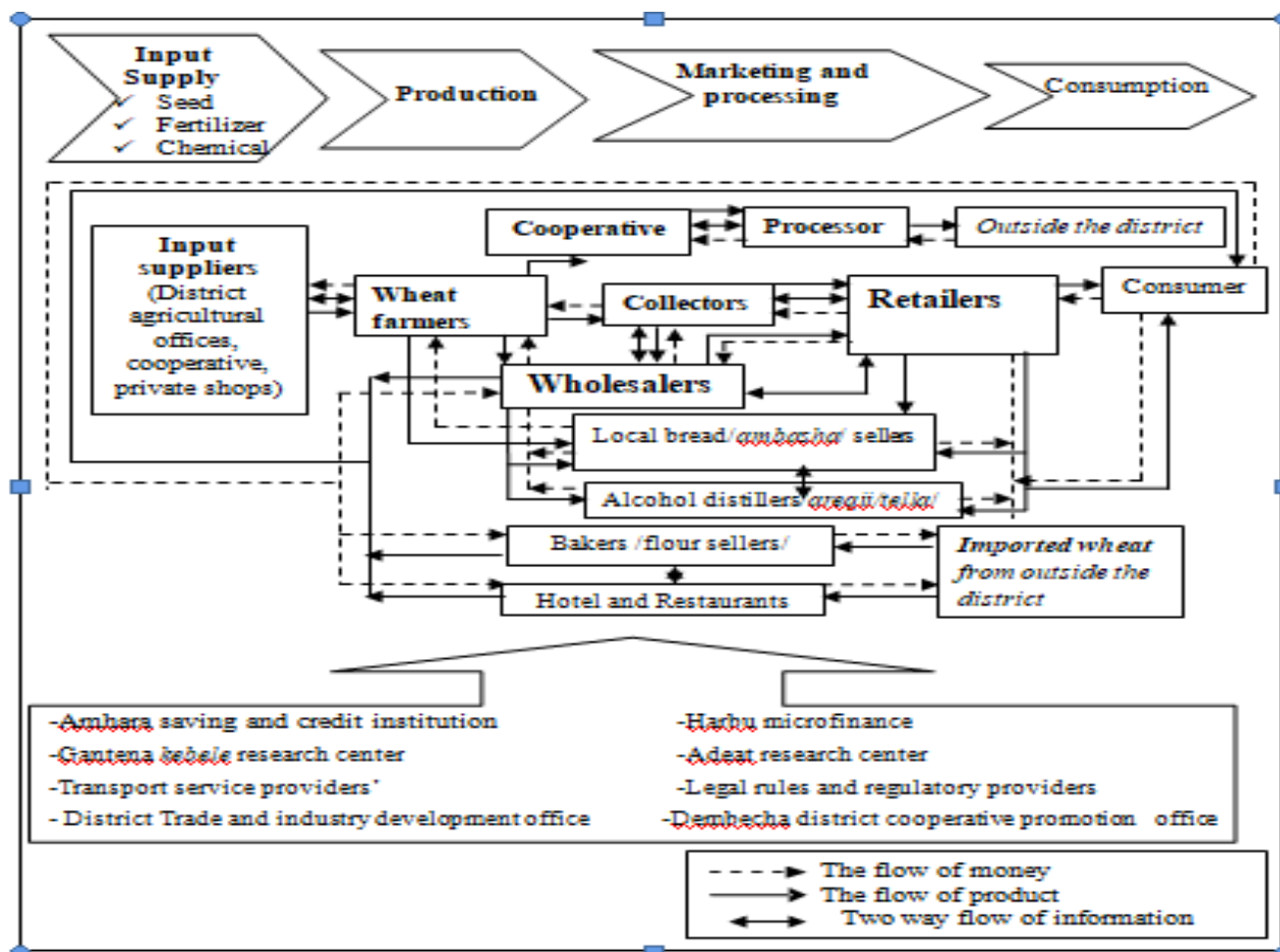


Figure 2: Value chain map for wheat

## Value chain actors and their roles

Value chain actors highlighted as those participants who are participated directly or indirectly in the value chain development of wheat. These actors may participated in either of input provision, production, marketing, processing, consuming or involved the one who involved in participating to the above listed activities. In this study, value chain actors of wheat include direct actors (input suppliers, producers, traders, consumers) and indirect actors are those that provide financial or non-financial support services, such as credit agencies, business service providers, government, cooperatives, researchers and extension agents.

**Input suppliers:** Input application is one of the most important farming activities that are used by wheat growers to produce and market in the study area. Among other input; seeds, fertilizer (Urea and DAP), chemicals (2, 4-D and Pallas) and farm implements are the major ones that wheat producers used to produce. These inputs were provided by different wheat value actors. Primary cooperatives, district agricultural developments office and individuals and private shops are the major input sources in the district. Cooperative unions of the zone indirectly participated input provision through its branch at district level.

**Wheat producers:** They are

smallholder farmers of the district who produce wheat for market and/or consumption. Wheat producers are important actors who perform most of the value chain functions right from farm inputs preparation on their farms or procurement of the inputs from other sources to post harvest handling and marketing. They are the main actors of the chain by participating both in the product market to supply their output and factor market in purchase basic inputs from input suppliers. Among others activities ploughing, sowing, fertilization; weeding, pest/disease controlling, harvesting, post-harvest handling and marketing are mainly undertaken by wheat farmers.

**Collectors:** These are actors in the wheat value chain who collects wheat from smallholder farmers in village markets or at the farm gate and reselling it to wholesalers and retailers in district market (Dembecha). Collectors facilitate transaction by serving as an intermediary among wheat producers, wholesalers and retailers. Buying, assembling, transporting, packing and selling to wholesalers and retailers are some of the important activities done by them.

**Wholesalers:** They are key actors in wheat value chain and participated in buying relatively large volume of wheat from collector or producers and selling it to retailers and consumers. Wholesalers have relatively strong working capital, better storage house, and communication access than any

other traders of the district. They govern the value chain of wheat in the study area as most of the time they determine quantity, price, quality of wheat that the upstream and downstream actors are selling or buying from them.

**Retailers:** Retailers delivers wheat to consumers. In the present study, they are all located in the district town. They own and handle wheat for a short period and incur relatively small marketing cost to the product. They purchase from collectors and wholesalers at district market and resale it to consumers. They have limited working capital and operate in small scale compared to other wheat traders of the district.

**Cooperatives/unions:** They play significant role for wheat value chain development by promoting producers to produce more by providing input and buying output at relatively good price. Most cooperatives in the study area located near the village market (*kebeles*) and this helps wheat farmers to become more informative on production and marketing strategy. Besides, the cooperatives also distribute sugar and oil mainly to members. They purchased from wheat farmers and resale to the union processors.

**Processors:** Millers/flour factory are also another wheat value chain actors that process wheat purchased from district cooperatives and distribute the processed flour to flour wholesalers, retailers, hotels/restaurants, bakers,

and also directly to consumers. It is located outside the district. Potential customers for the processors are also found in different parts of the zone.

**Local alcohol processors:** They are important value chain actors that purchase wheat from retailers and wholesalers and process wheat in to local alcohol which is locally named as *areqi* and *tella*. Buying, grading, sorting, milling, processing and selling alcohol is the most important task of alcohol processors. Local alcohol processing recently becomes as an important business that creates multi-dimensional job opportunities like fattening of oxen.

**Local bread/ ambasha/ sellers:** Those actors engaged in the marketing of bread prepared from wheat. They purchased from wheat grower farmers, retailers and wholesalers.

**Hotels/Restaurants/Bakers:** They are also actors of wheat value chain that take wheat flour from processors or flour sellers outside the district and provide to district consumer. Those groups of respondents were complaining the absence of flour factory near to them and the main reason for the relatively high cost of bread and other wheat products.

**Consumers:** They are the end actors in wheat value chain. Wheat is consumed in the form of bread, *kollo*, *enjera* and alcohol (*tella*, *areqi*). Therefore, the marketed wheat reaches consumers through direct purchasing from farmers or from wholesalers,

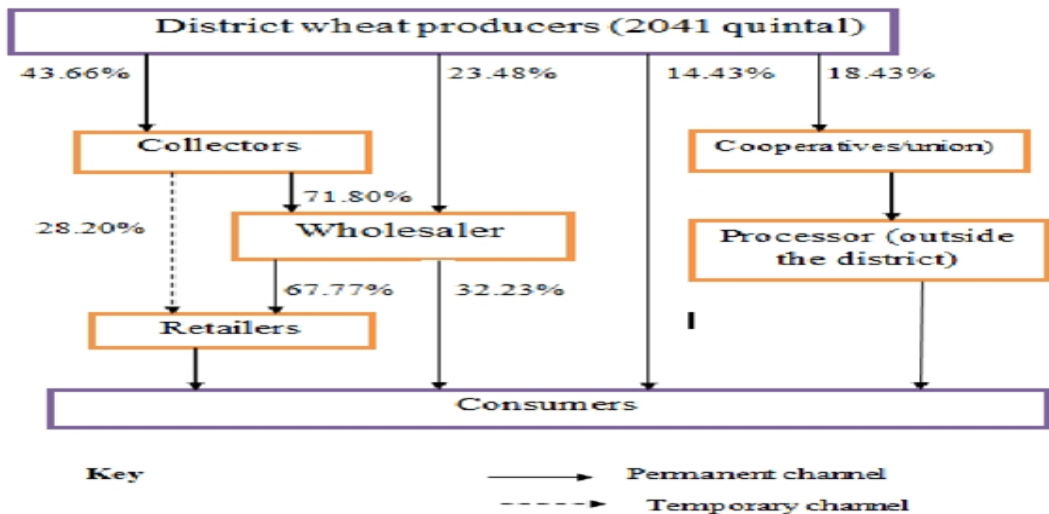
retailers, hotels and restaurants as food and alcohol. Residents of the rural and urban, peoples visiting markets, travelers, etc. are the major consumers of wheat and wheat product in the study area.

**Chain supporters/enablers:** Those are either individual or institution that are engaged either in financing or supporting main actors of wheat value chain to perform different value chain activities. They do not participate directly in the wheat value chain, but indirectly facilitate major actors in wheat in the chain to make their task effective and practical. District agricultural and natural development office, cooperative promotion office, micro finance (Harebu micro finance), Amhara Saving and Credit Institution,

district trade and market development office, transport service providers are the major supportive institutions playing a central role in the provision of such services.

### Marketing channels of wheat

Wheat market channel for this study were designed based on volume of flow of product (wheat) passing through different routes during the 2016 /17 cropping season. Seven wheat-marketing channels were identified to deliver the product from producer to consumers. A total of 3516 quintals of wheat was produced by the sampled wheat producers' households. As depicted below, the major identified channels from the survey were listed as follows:



## **Structure-conduct and performance of wheat market**

### **Structure of wheat market**

Market concentration, degree of transparency (market information) and entry conditions such as licensing procedure, lack of capital and managerial know how was used to evaluate wheat market structure.

### **Four firms' concentration ratio:**

CR4 for four largest firms at district level was found to be 39.78. This implies wheat market in the study area is characterized by weak oligopoly market structure, indicating the existence of market imperfection. Four wheat traders handled annually 39.78 % of the total volume of wheat purchased by the sample traders and thereby wheat market at district level is inefficient and noncompetitive.

### **Market Transparency (information):**

the availability of accessible and timely information on price is a crucial for market participants. All wheat traders were get market information, but wheat producers were affected by lack of clear and reliable market information. Focus group discussion and key informants support the existence of information asymmetry among participants. This implies the market deviates from competitive norms as result of poor information flow.

**Barriers to entry:** Capturing data from traders were difficult due to the

reason that sampled traders are already in the business. However, dealing on traders characteristics like educational level, trade experience, working capital requirement and legal licenses was considered to get such information. Education background, working capital and license were barriers to enter wheat market. The focus group discussion and key informants showed that a means acquiring customer is one barrier to enter in wheat trade business.

### **Conduct of wheat market**

The agricultural loan collection schedule, governmental taxes, the existence of many religious and cultural festivals and the easement time for farmers following the harvest season makes farmers to supply more and traders to cut the market prices. Moreover, distance of the market from their residence, absence of storage house near to market center and competition among traders are some of the determinants that makes traders in unusual way in the study area. Therefore, conduct of wheat market showed wheat traders behave in unusual way to maximize their profit.

### **Performance of the wheat market**

The performance of wheat market was evaluated by considering associated costs, returns and marketing margins. The Ethiopian Birr (ETB) per quintal was used as a standardized unit of measurement for performance analysis.

**Marketing cost:** wheat producers incur production costs for production prior to marketing. Most households used their family labor, oxen and land for wheat production, opportunity costs were used to compute costs of production. The average cost of wheat production was 375.63 Birr/qt, of which 37.6 percent were spent for purchasing inputs and about 31.68 percent costs were used for land rent.

The processors cost is highest (ETB 156.60 per quintal) from other actors. This is may be due to additional cost for processing. The cost is followed by cooperatives (59.50 Birr/qt) and collectors (41.71 Birr/qt) as they undertake more value adding activities as they directly received from farmers and needs more transportation cost to transport to their potential customers. Similarly, the structure of marketing cost revealed that transportation cost is the highest cost for all other market actors except processors.

**Marketing margin:** The marketing margin of an agent was calculated using the average price of wheat for that particular agent. However, there

are situations that one market agent sold at different price to the next actors. The result from focus group discussion and key informants interview also showed that quantity, transportation cost and quality are among the important factors that were considered to differentiate price.

Total gross marketing margin (TGMM) is highest in channel VII (46.62 percent) and followed by channel VI and II which accounts for 17.72 percent of the consumer price and lowest which accounts 8.06 percent of consumer prices and producer share (GMMp) is uppermost (91.94 percent) of consumer price in channel V. This result shows as number of marketing agents decreases the producers share increases and vice versa. The higher number of middlemen in the commodity market, the more profit they retain for their services. However, the existence of a high market share does not necessarily mean that the market is performing well and wheat traders or the smallholder producers are getting fair benefits from the wheat marketing.

Table 1: Wheat marketing margin for different channels (Birr/qt)

| Channel | GMMp  | GMM col | GMM cop | GMMpr | GMMwho | GMMrt | TGGM  |
|---------|-------|---------|---------|-------|--------|-------|-------|
| I       | 100   |         |         |       |        |       | 0.00  |
| II      | 82.28 | 7.34    |         |       |        | 10.38 | 17.72 |
| III     | 85.63 | 6.58    |         |       | 7.79   |       | 14.37 |
| IV      | 82.28 | 6.32    |         |       | 6.90   | 4.49  | 17.72 |
| V       | 91.94 |         |         |       | 8.05   |       | 8.06  |
| VI      | 88.35 |         |         |       | 7.45   | 4.49  | 11.65 |
| VII     | 53.38 |         | 6.88    | 39.72 |        |       | 46.62 |

Moreover, processors obtain relatively highest gross marketing margin (39.72%) of consumer price in channel VII followed by retailers which was 10.38 % in channel II among wheat traders. Retailers also obtain lowest market margin (4.49%) from other traders as they purchase from wholesalers. This implies share of market intermediaries from consumers' price was substantial and thereby it reduces producers' share from consumers' price.

**Profitability Analysis::** The profitability analysis results showed that wheat producers' market profit was highest when they directly sell to consumers in channel I which is 265.62 birr/qt and cooperatives in channel VII which is about 258.49 birr/qt while taking lowest market profit when they sell to collectors which accounts 234.27 birr/qt. Among traders processors shared the highest profit 339.97 birr/qt in channel VII. Retailers gained the second highest profit of 60.44 birr/qt from channel II, when they bought from collectors and sold to consumers. The profitability analysis showed that all wheat market actors are profitable. Cost-benefit analysis revealed that traders receive relatively high profit by adding relatively small cost. Additionally, producers are relatively disadvantaged from wheat market due to disproportionate share of profit relative to cost. The result is in line with study by Abrham (2009).

## Conclusion and Recommendation

Value chain actor's relationship, information flow, pie growing mechanisms is very weak among value chain of wheat in the study area. Traders and producers did not cooperate and work together to enhance production rather they try to cheat each other to maximize their own benefit. This makes wheat value chain to be ineffective in the study area. The structure-conduct-performance paradigm of wheat market in the study area appeared to be poor structured (weak oligopoly), and deviating from competitive norms. Due to this reason producers are the most disadvantaged from wheat market and thereby the market area is not performing well.

Based on the finding of this study the following recommendations are necessary drawn: facilitate agro-processing establishment, supporting farmers, increase production through practical research, providing pie-growing mechanisms, increase farmers bargaining power and providing infrastructural facilities, designing advance way of disseminating market information, determining appropriate pricing strategies based on true costs of production should be done by government or any other stakeholder to strength the value chain of wheat in the study area.

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# Registration Of 'Walin' (BG-004-1) Sesame (*Sesamum indicum* L.) Variety

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## Abstract

The name Walin was given to sesame variety developed and released by Bako agricultural research center of Oromia agricultural research institute in the year 2017. It is selected from landraces collection made in Benishangul Gumuz area after pure and homogenous line is developed from population with accession number BG004-1. This variety gave high grain yield with 22.2% advantage over the standard check, Chalasa and has high oil content (55.1%). The GGE-biplot analysis also indicated that this variety is stable and gave high yield. Furthermore, Walin is tolerant to bacterial blight, relatively larger seed size, white seeded and erect growth habit. It was released for Uke, Chewaka and Angare Gute areas and similar agro ecologies.

**Keywords:** *Sesamum indicum*, Variety Verification, Variety Release

## Introduction

Sesame (*Sesamum indicum* L.,  $2n = 26$ ), which belongs to the genus *Sesamum* and the family Pedaliaceae, is one of the oldest oilseed crops and cultivated in tropical and subtropical regions of Asia, Africa and South America (Zhang *et al.*, 2013). Sesame is the first ranking export oilseed crop in Ethiopia. It is cultivated by small and large scale farmers, and private investors. There is huge gap between the average national grain yield which is less than  $1 \text{ t ha}^{-1}$  and the genetic potential of sesame in Ethiopia. This was mainly due to shortage of improved sesame varieties (Negash,

2015). Western Ethiopia is characterized as high rainfall areas where bacterial blight is the bottle neck for sesame production. Due to high disease pressure, only three varieties were adapted to western part of Ethiopia among a number of sesame varieties released at national level. Given the fact that western Ethiopia is one of the potential areas for sesame production (FAO, 2015), the demand for adaptable improved varieties of this crop is very high. Therefore, developing a new variety with high yield and desirable agronomic traits is the main objectives of sesame improvement program. Therefore, there is a need to identify high

yielding and stable sesame variety with disease resistance for the western part of Oromia.

## Varietal Origin and Evaluation

Walin (BG-004-1) was selected out of landraces population collected from Benishangul Gumuz regional state, western Ethiopia. Including Walin and the standard check, fifteen sesame genotypes were evaluated in sesame regional variety trial at Uke and Chewaka for two years (2014 and 2015) and at Gutin and Mender-10 for one year (2015).

## Agronomic and morphological characteristics

The released sesame variety 'Walin' has acceptable marketable seed size and thousand seed weight (3 g), erect growth habit which is very important trait, white seed color and it is tolerant to major sesame diseases prevailing in the areas. The detail description of the released variety is presented in Table 1.

Table 1. Agronomic and morphological characteristics of new sesame variety 'Walin'

|   |                                             |                                                |
|---|---------------------------------------------|------------------------------------------------|
| 1 | Variety                                     | Walin (BG-004-1)                               |
| 2 | Agronomic & morphological characters        |                                                |
|   | 2.1. Adaption area:                         | Chewaka, Uke, Gutin and similar agro ecologies |
|   | Altitude (ma.s.l)                           | 1250-1450                                      |
|   | Rain fall (mm)                              | <750                                           |
|   | 2.2. Seed rate (kg ha <sup>-1</sup> )       | 5-10                                           |
|   | 2.3. Spacing (cm)                           | 40x5 (Inter and Intra rows)                    |
|   | 2.4. Planting date                          | Late May to early June                         |
|   | 2.5. Fertilizer rate (kg ha <sup>-1</sup> ) | 100 NPS and 50 UREA                            |
|   | 2.6. Days to flowering                      | 54-68                                          |
|   | 2.7. Days to maturity                       | 80-110                                         |
|   | 2.8. Plant height (cm)                      | 120-160                                        |
|   | 2.9. 1000 seed weight (g)                   | 3                                              |
|   | 2.10. Seed color                            | Dull white                                     |
|   | 2.11. Growth habit                          | Erect                                          |
|   | 2.12. Crop pest reaction                    | Tolerant to major sesame diseases              |
|   | 2.14. Yield (t ha <sup>-1</sup> )           |                                                |
|   | Research field                              | 1.0-1.38                                       |
|   | Farmers field                               | 9.5-11                                         |
| 3 | Year of release                             | 2017                                           |
| 4 | Breeder seed maintainer                     | Bako agricultural research center              |

## Yield Performance

Walin was evaluated in multi-location trial with thirteen pipe line varieties against one standard check, Chalasa for two years (2014 and 2015). It gave grain yield varying 1.0-1.38 t ha<sup>-1</sup> on station (Table 2). The on farmers' field grain yield performance of Walin was ranged from 9.5-11qt/ha.

## Adaptation and agronomic recommendations

Newly released sesame variety, Walin is recommended for Uke, Chewaka and Gutin and areas with similar agro-ecologies of Ethiopia. It is well adapted in altitude ranging from 1250 to 1450 m.a.s.l. with annual rainfall of

750 mm. Recommended seed rate is 5 kg ha<sup>-1</sup> while fertilizer rate is 100 kg ha<sup>-1</sup> NPS at planting and 50 kg ha<sup>-1</sup> Urea 30 days after planting. The recommended planting time ranging from late May to early June depending on the onset of the rain fall.

## GGE-Biplot analysis

### The ranking of genotypes based on yield and stability

Both PC1 and PC2 were separated based on their scored for fifteen sesame tested genotypes in the study area. The designated genotypes code and Environments code were listed in the table below.

Table 2. Genotypes and environments and their codes

| No | Genotype      | Genotype code | No | Environments | Env. code |
|----|---------------|---------------|----|--------------|-----------|
| 1  | BG-005        | G1            | 1  | Uke 2014     | E1        |
| 2  | EW-009        | G2            | 2  | Uke 2015     | E2        |
| 3  | EW-022        | G3            | 3  | Chewaka 2014 | E3        |
| 4  | EW-023        | G4            | 4  | Chewaka 2015 | E4        |
| 5  | BG-004-1      | G5            | 5  | Gutin        | E5        |
| 6  | EW-007        | G6            | 6  | Mender 10    | E6        |
| 7  | EW-021        | G7            |    |              |           |
| 8  | EW-005-1      | G8            |    |              |           |
| 9  | EW-009-1      | G9            |    |              |           |
| 10 | EW-008        | G10           |    |              |           |
| 11 | WW-005-1      | G11           |    |              |           |
| 12 | IL-003        | G12           |    |              |           |
| 13 | BG004(1)01BK  | G13           |    |              |           |
| 14 | EW011(2)-01BK | G14           |    |              |           |
| 15 | Chalasa       | G15           |    |              |           |

GGE-biplot is mainly used to identify the scaling of genotypes for the locations. As (Yan et al., 2000; Yan, 2002) reported, when PC1 in GGE-biplot approximates the genotype (mean performance), PC2 must

approximate the G x E associated with each genotype which is the measure of stability or instability. Genotypes having PC1 > 0 were recognized as high yielding while those genotypes

having PC1 score  $< 0$  were identified as low yielding, (Kaya *et al.* 2006).

Genotypes 2 (EW-009), 5 (BG-004-1), 11 (WW-005-1), 12 (IL-003) and 14 (EW011 (2)-01BK ), are more stable

and give higher yield and Environment 2, 5 and 6 also ideal for the production of this particular crop sesame. The stability and GGE- biplot diagrams are sketch below.

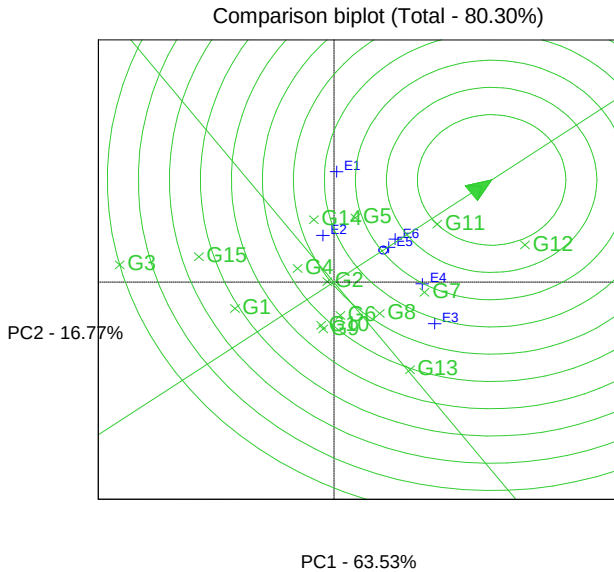


Figure 1. GGE-biplot based on genotype focused scaling for comparison of the genotypes

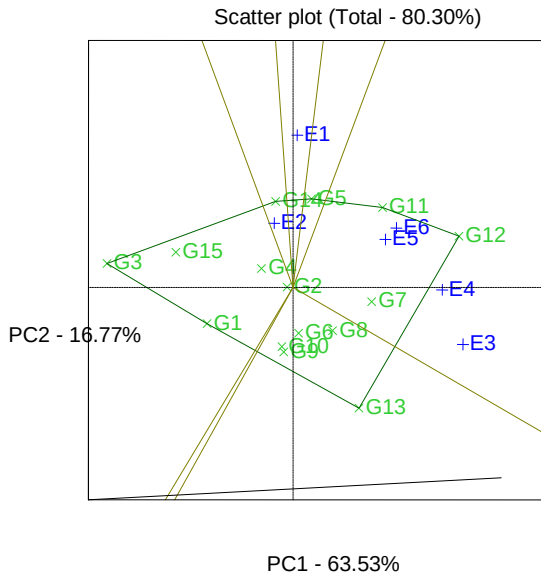


Figure 2. The polygon view of the GGE- biplot based on symmetrical scaling for genotypes and environments. PC, G and E stands for principal component, genotype and environments, respectively.

Table 3. Mean seed yield performance of sesame genotypes across locations and years.

| Pipe line variety | Grain (kg ha <sup>-1</sup> ) |               |              |               |              |               | mean          | Yield advantage (%) |
|-------------------|------------------------------|---------------|--------------|---------------|--------------|---------------|---------------|---------------------|
|                   | 2014                         |               |              | 2015          |              |               |               |                     |
|                   | Chewaka                      | Uke           | Gutin        | Chewaka       | Uke          | Mender 10     |               |                     |
| BG-005            | 892.4                        | 987.2         | 510.9        | 1016.1        | 893.7        | 1038          | 889.7         | -0.19               |
| EW-009            | 1093.2                       | 1059.5        | 551          | 1229.7        | 1058.3       | 1150          | 1023.6        | 14.8                |
| EW-022            | 512.2                        | 1060.2        | 420.3        | 911.5         | 981.7        | 965.6         | 808.5         | -9.3                |
| EW-023            | 1054.5                       | 1112.8        | 575.5        | 1040.6        | 867.7        | 1210.4        | 976.9         | 9.6                 |
| <b>BG-004-1</b>   | <b>1054.8</b>                | <b>1294.3</b> | <b>843.2</b> | <b>1258.9</b> | <b>971.8</b> | <b>1114.6</b> | <b>1089.6</b> | <b>22.2</b>         |
| EW-007            | 1098.5                       | 955.8         | 611.9        | 1286.5        | 954.7        | 1096.4        | 1000.6        | 12.2                |
| EW-021            | 1244.6                       | 1023.4        | 665.6        | 1396.4        | 918.2        | 1331.8        | 1096.6        | 23                  |
| EW-005-1          | 1138.2                       | 918.7         | 667.7        | 1283.3        | 871.3        | 1297.4        | 1029.4        | 15.4                |
| EW-009-1          | 1012.2                       | 850           | 771.3        | 1055.7        | 713          | 1250          | 942.0         | 5.6                 |
| EW-008            | 1063.2                       | 933.7         | 703.1        | 1133.9        | 784.9        | 1107.8        | 954.4         | 7                   |
| WW-005-1          | 1206.6                       | 1235.6        | 842.2        | 1377.1        | 951.5        | 1364.1        | 1162.8        | 30.4                |
| IL-003            | 1366.6                       | 1198.7        | 904.2        | 1583.9        | 841.1        | 1450          | 1224.0        | 37.3                |
| BG-004(1)01BK     | 1324.3                       | 892.7         | 683.8        | 1386.5        | 714          | 1046.9        | 1008.0        | 13                  |
| EW-011(2)-01BK    | 858.8                        | 1160.4        | 774.5        | 1082.3        | 866.7        | 1401.6        | 1024.0        | 14.8                |
| Chalasa(EW-023)   | 823.7                        | 1255.8        | 536.9        | 976.6         | 878.1        | 877.6         | 891.4         | 0                   |
| Grand mean        | 1049.5                       | 1062.5        | 670.8        | 1201.2        | 884.4        | 1180.1        | 1049.5        | 13                  |
| CV (%)            | 13.2                         | 15.2          | 14.2         | 13.7          | 10.1         | 13.6          |               |                     |
| LSD (5%)          | 231.3                        | 271.1         | 159.2        | 274.5         | 149.4        | 269.2         |               |                     |
| F – value         | **                           | **            | **           | **            | **           | **            |               |                     |

**Quality Attributes and reaction to disease**

As compared to the candidates and the check, Walin (BG-004-1) was the best variety with oil content of 55.1% (Table 3). Besides, Walin is moderately resistant to bacterial blight.

Table 4. Oil content and disease reaction of sesame varieties evaluated under verification trial

| No | Variety          | Oil content (%) | Reaction to bacterial blight (1-9 scale) |
|----|------------------|-----------------|------------------------------------------|
| 1  | BG-004-1 (Walin) | 55.1            | 3.75                                     |
| 2  | WW-005-1         | 52.6            | 3.75                                     |
| 3  | IL-003           | 54.2            | 5                                        |
| 4  | Chalasa (check)  | 52.7            | 5                                        |

NB: disease score Where: 1 - 3 = resistance; 4 = moderately resistant; 5 - 6 = moderately susceptible; 7 - 8 = susceptible 9 = highly susceptible

## Conclusion

Walin variety was officially released in April 2017 for its high and stable grain yield performance, tolerance to major sesame diseases, high oil content, acceptable seed size, and early maturing with an erect growth habit. This variety is recommended for production in Uke, Chewaka, Angar Gute and areas with similar agro ecologies.

## Acknowledgements

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# Registration of “Dursi” Newly Released Tef (*Eragrostis tef* (Zucc.) Trotter) Variety

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## Abstract

The name **Dursi** was given to tef [*Eragrostis tef* (Zucc.) Trotter] variety with the pedigree of Acc 236952 primarily collected by the Ethiopian Biodiversity Institute from Arsi zone, Dodota Sire district. **Dursi** was evaluated against standard check (**Kena**), eight other pipeline varieties and a local check at Shambu, Gedo and Arjo sub sites during 2016 and 2017 main cropping season. Yield stability analyses using Genotype and Genotype by Environment interaction (GGE) biplot, revealed that **Dursi** is stable and high yielding (2285 kg ha<sup>-1</sup>) with 26% yield advantage over the standard check and stable performance in western Oromia. Therefore, **Dursi** was fully released in 2018 for wider production in the highlands of Western Oromia and similar agro-ecologies.

**Keywords:** *Eragrostis tef*, Genotype and Genotype by environment interaction (GGE), Stability

## Introduction

*Eragrostis tef* (Zucc.) Trotter, is a self-pollinated warm season annual grass with the advantage of C4 photosynthetic pathway (Seyfu, 1997). Tef is among the major Ethiopian cereal crops grown on more than 3 million hectares annually (CSA, 2017), and serving as staple food grain for over 70 million people. Tef has an attractive nutritional profile, being high in dietary fiber, iron, calcium and carbohydrate (Hager *et al.*, 2012). Besides, it has high level of phosphorus, copper, aluminum, barium, thiamine and excellent composition of amino acids essential

for humans (Abebe *et al.*, 2007). The straw (*chid*) is an important source of feed for livestock. Tef is also a resilient crop adapted to diverse agro-ecologies with reasonable tolerance to both low (especially terminal drought) and high (water logging) moisture stresses. Tef, therefore, is useful as a low-risk crop to farmers due to its high potential of adaptation to climate change and fluctuating environmental conditions (Balsamo *et al.*, 2005). Nevertheless, tef was considered as “orphan” crop: the one receiving no international attention for research and other technologies applicable to smallholder farmers (Seyfu, 1997). Because of its gluten-free proteins and slow release carbohydrate constituents,

tef is advocated and promoted as health crop at global level (Spaenij-Dekking *et al.*, 2005). Inadequate research investment to improvement of the crop is one among the major tef productivity constraints. Therefore, the objective of this activity was to evaluate and release high yielding, lodging and diseases tolerant tef variety for tef growing areas of western parts of the country.

### Variety origin and evaluation

*Dursi* (Acc. 236952) was formerly collected by the Ethiopian Biodiversity Institute (EBI) from Arsi zone, Dodota Sire district. It was primarily selected from the 81 tef landraces evaluated for MSc thesis research (Dagnachew *et al.*, 2008) and promoted to the succeeding breeding steps through single plant selection method. A total

of nine pipeline tef genotypes were evaluated at Shambu, Gedo and Arjo research sub sites against standard (*Kena*) and local check for two consecutive years (2016 and 2017). *Dursi* was selected and verified in 2018 for its high grain yield and stable performance across locations and years.

### Morphological and Agronomic characteristics

*Dursi* has medium plant height, good tillering capacity and tolerant to lodging. Detail description of the variety is presented in Table 1 and Table 2. Combined analysis of variance revealed highly significant ( $p < 0.01$ ) difference among genotypes for plant height, panicle length, shoot biomass, lodging % and grain yield (Table 1).

Table 1. Mean grain yield (kg ha<sup>-1</sup>) per location across years

| Genotype    | Shambu  |         | Gedo    |         | Arjo    |         | Mean | % yield advantage |
|-------------|---------|---------|---------|---------|---------|---------|------|-------------------|
|             | 2015/16 | 2016/17 | 2015/16 | 2016/17 | 2015/16 | 2016/17 |      |                   |
| Acc.236952  | 2507    | 2120    | 2256    | 2330    | 2134    | 2363    | 2285 | 26                |
| Acc.55253   | 2187    | 2302    | 2195    | 2181    | 2012    | 2181    | 2176 | 19.29             |
| DZ-01-1001  | 1916    | 2061    | 1703    | 1858    | 1675    | 1887    | 1850 |                   |
| DZ-01-1004B | 1931    | 2042    | 1653    | 1677    | 1672    | 1652    | 1771 |                   |
| DZ-01-102   | 2180    | 2030    | 1900    | 2010    | 2074    | 1969    | 2027 | 11.13             |
| DZ-01-385   | 2044    | 1882    | 1871    | 2102    | 1477    | 2081    | 1910 |                   |
| DZ-01-739   | 1922    | 1997    | 1943    | 1848    | 1755    | 1841    | 1884 |                   |
| DZ-01-778   | 2065    | 1902    | 2002    | 1800    | 1853    | 1883    | 1918 |                   |
| DZ-01-821   | 2018    | 1894    | 1938    | 1851    | 1831    | 1914    | 1908 |                   |
| Kena        | 2009    | 2043    | 1830    | 1637    | 1783    | 1644    | 1824 |                   |
| Local       | 1691    | 1798    | 1748    | 1806    | 1706    | 1777    | 1754 |                   |
| Mean        | 2025    | 2043    | 1918    | 1927    | 1816    | 1927    | 1937 |                   |
| CV          | 8.9     | 6.3     | 6.6     | 6.1     | 11.3    | 4.3     |      |                   |
| F-Value     | <0.005  | <0.002  | <0.001  | <0.001  | <0.028  | <0.001  |      |                   |

Table 2: Mean Agronomic traits across years and locations

| Genotype    | GY (t ha <sup>-1</sup> ) | LD%   | LR    | NFT   | PH    | PL    | SBM (t ha <sup>-1</sup> ) |
|-------------|--------------------------|-------|-------|-------|-------|-------|---------------------------|
| Local check | 1.75                     | 60.56 | 1.00  | 16.34 | 40.19 | 24.67 | 66.21                     |
| DZ-01-1004B | 1.77                     | 56.67 | 3.42  | 17.97 | 43.03 | 29.53 | 79.79                     |
| Acc.236952  | 2.29                     | 6.89  | 1.50  | 18.88 | 45.24 | 34.13 | 87.71                     |
| DZ-01-821   | 1.91                     | 10.00 | 2.76  | 18.33 | 51.41 | 32.87 | 67.36                     |
| Acc.55253   | 2.18                     | 13.61 | 1.60  | 20.91 | 49.02 | 33.07 | 89.56                     |
| Kena        | 1.82                     | 79.44 | 2.00  | 18.38 | 45.58 | 29.67 | 82.54                     |
| DZ-01-739   | 1.88                     | 6.89  | 1.41  | 21.24 | 56.07 | 27.47 | 87.75                     |
| DZ-01-1001  | 1.85                     | 18.89 | 3.39  | 18.32 | 50.99 | 32.07 | 62.71                     |
| DZ-01-102   | 2.03                     | 60.00 | 1.61  | 17.89 | 47.00 | 33.53 | 79.82                     |
| DZ-01-385   | 1.91                     | 7.67  | 3.56  | 20.43 | 50.54 | 26.80 | 86.17                     |
| DZ-01-778   | 1.92                     | 43.33 | 3.11  | 20.56 | 51.91 | 30.20 | 84.64                     |
| Mean        | 1.94                     | 32.85 | 2.31  | 19.02 | 48.27 | 30.36 | 79.80                     |
| CV%         | 6.70                     | 34.00 | 26.40 | 14.10 | 10.30 | 8.30  | 18.90                     |
| LSD         | 0.09                     | 8.66  | 0.75  | 1.77  | 3.28  | 3.49  | 9.97                      |
| F-Value     | **                       | **    | **    | **    | **    | **    | **                        |

Key: GY (t ha<sup>-1</sup>)=Grain yield ton per hectare, LD%=Lodging %, LR=leaf rust, NFT=Number effective tiller, PH=plant height, PL=Panicle Length, SBM (t ha<sup>-1</sup>)=Shoot Biomass ton per hectare

## Yield potential, its Stability and Adaptability

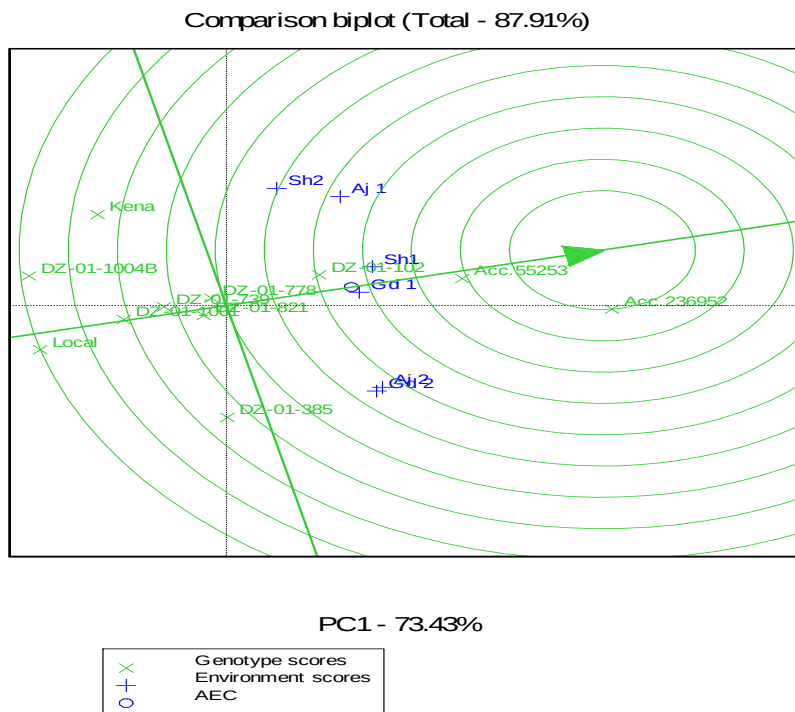
The new variety *Dursi* showed higher mean grain yield (2285 kg ha<sup>-1</sup>) and more stable performance with yield advantage of 26% over the standard check, *Kena* (1824 kg ha<sup>-1</sup>) and the local check (1754 kg ha<sup>-1</sup>). Results from GGE biplot analysis also showed that *Dursi* (Acc 236952) fall in the central circle away from vertical mean line and closer to the origin, indicating its high yield potential and relative

stability and wider adaptability compared to the other genotypes (Fig.1). *Dursi* was released for the highlands of Western Oromia and similar agro-ecologies receiving sufficient amount of rainfall (1800 mm-2000 mm) and altitude ranges of 1850-2500 m.a.s.l. The variety performs best with its full agronomic recommendations presented in Table 1.

Table 1. Agronomic &amp; morphological characteristics of Dursi variety

| Agronomic characters and descriptions of <i>Dursi</i> |                                                                                                                                                  |
|-------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Variety name                                          | <i>Dursi</i> (Acc. 236952)                                                                                                                       |
| Adaptation area                                       | Shambu, Gedo, Arjo, and similar agro ecologies                                                                                                   |
| •Altitude (masl)                                      | 1850-2500                                                                                                                                        |
| •Rainfall (mm)                                        | 1800-2000                                                                                                                                        |
| Seeding rate (kg ha <sup>-1</sup> )                   | 10 and 15 (row planting and broad cast, respectively)                                                                                            |
| Spacing (cm):                                         | 20 cm between rows                                                                                                                               |
| Planting date:                                        | Early to mid-July                                                                                                                                |
| Fertilizer rate (kg ha <sup>-1</sup> ):               | <ul style="list-style-type: none"> <li>•100 DAP at planting</li> <li>•50 UREA (half at planting &amp; half after 25 days)</li> </ul>             |
| Days to heading:                                      | 70                                                                                                                                               |
| Days to maturity:                                     | 132                                                                                                                                              |
| 1000 seed weight (g):                                 | 0.3                                                                                                                                              |
| Plant height (cm):                                    | 115                                                                                                                                              |
| Seed color:                                           | cream White                                                                                                                                      |
| Panicle color:                                        | yellowish at maturity                                                                                                                            |
| Crop pest reaction*                                   |                                                                                                                                                  |
| Grain yield (t ha <sup>-1</sup> ):                    | <ul style="list-style-type: none"> <li>•On farmers field: 1.8-2.2 t ha<sup>-1</sup>.</li> <li>•On-station: 2.0-2.4 t ha<sup>-1</sup>.</li> </ul> |
| Year of release:                                      | 2018                                                                                                                                             |
| Breeder/ maintainer:                                  | BARC/OARI                                                                                                                                        |

\*=Tolerant to major Tef diseases (Head smudge and Rust)



Key: SH1 and SH2=Shambu year one and two, Gd1 and GD2= Gedo year one and two, Aj1 and Aj2=Arjo year one and two

Fig 1: GGE biplot analysis showing stability of genotypes and test environments

## Conclusion

*Dursi* is stable in its grain yield and has good agronomic traits that make it suitable for production in the high highlands of western Oromia and similar agro-ecologies.

## Acknowledgements

This study was supported by the Oromia Agricultural Research Institute (IQQO) Agricultural Growth Program (AGP-II). Staff members of Bako Agricultural Research Center in general and cereal crop research team in particular are acknowledged for their support in the development of this variety.

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# Registration of “Bulala” a Newly Released Durum Wheat (*Triticum durum* L.) Variety

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## Abstract

*Bulala is a name given to a newly released durum wheat variety released by Sinana Agricultural Research Center in 2016/17 from a selection made among durum wheat germplasm introduced from ICARDA. Seventeen durum wheat genotypes were tested along with three standard checks for two years (2012 and 2013) at four locations (Sinana, Agarfa, Ginir and Gololcha), highlands of Bale. The combined analysis of yield data across years and locations showed that, Bulala gave highest mean yield (5641.5 kg ha<sup>-1</sup>) as compared to the remaining test entries. Moreover, Bulala showed more stable grain yield performance, disease resistance, uniformity, high protein and gluten content, , high biomass and also early maturing than checks (Tate, Toltu and Ingilize). During variety verification trial, this variety was the most preferred varieties by the farmers as compared to the checks in the trial. It was evaluated both on farmers field and on research station during variety verification trial along with checks (Dire and Ingilize) in 2016 cropping season and was officially released in 2017 for durum wheat growing areas of Bale and similar agro-ecologies.*

**Keywords:** Durum Wheat (*Triticum durum*), Grain Quality, Protein Content

## Introduction

Durum wheat (*Triticum durum* L.) is the second most cultivated wheat in the world next to bread wheat (Peña and Pfeiffer, 2005). It is indigenous to Ethiopia and it has been under cultivation since ancient times. It is traditionally grown on heavy black clay soils (Vertisols) of the central and northern highlands of Ethiopia between 1800-2800 meters above sea level. Durum is the hardest of all

wheat. Its density, combined with its high protein content and gluten strength makes it the preferred choice for producing pasta products. Pasta made from durum wheat is firm with consistent cooking quality. The dough is less elastic than bread dough's, but this makes it easier to roll into pasta shapes. Semolina of durum wheat is the preferred raw material for the production of high quality pasta due to its unique color, flavor and cooking quality. Pasta is a popular wheat-based food worldwide, due to its

convenience, palatability and nutritional value (Aravind *et al.*, 2011).

In Ethiopia, durum wheat is consumed as leavened bread, common bread, macaroni, spaghetti, biscuits, pastries, and in various indigenous food preparation. The straw is mainly used for cattle feed and for fuel at times of scarcity (MANR, 2016). However, the productivity of durum wheat in the country is very low compared to world average. This is due to lack of improved variety, low cultural practices, moisture stresses, and other biotic and abiotic factors. Rust is among the major problem limiting durum wheat productivity in Ethiopia. Thus, it is crucial for breeders to develop and recommend the right variety for commercial production in the target agro ecologies tolerant to prevailing stresses.

### **Variety origin and evaluation**

*Bulala* was originally introduced from abroad through ICARDA. A number of durum wheat genotypes along with checks were tested at different breeding stages starting from observation nursery. Seventeen pipeline durum wheat varieties were evaluated against three standard checks (Tate, Toltu and Ingilize) for two years (2012 and 2013) at Sinana, Agarfa, Ginir and Gololcha of Bale highland.

### **Agronomic and morphological characteristics**

*Bulala* has medium plant height (82cm) with good tillering capacity, lodging resistance, erected growth habit, large ear size, lodging resistance. Details of agronomic characters of *Bulala* is presented in Table 1.

### **Yield Performance and stability**

The combined yield data of the two years across four locations showed that, *Bulala* was the best high yielding variety with mean grain yield of 5641.5 kg ha<sup>-1</sup>. *Bulala* showed stable yield performance across years and locations. Besides, it has 16.9% and 25% yield advantage over two checks viz., Toltu and Englize, respectively (Table 2).

### **Quality Characteristics**

*Bulala* has higher protein (13.3%) and gluten (30.3%) content than the standard check, indicating that it has good pasta making quality (Table 3). Its seed color is also good and preferred by consumers.

### **Reaction to diseases**

The major durum wheat disease according to their importance in the growing area is rusts (yellow, stem and leaf rusts). *Bulala* showed 5mr % reaction to both stem and yellow rusts and *trms* % to leaf rust during verification trial (Table 3). Over all,

this variety is resistance to the major wheat rusts as compared to the checks and hence farmers in durum wheat growing areas of the highlands of Bale can use this variety to reduce the effect of rust epidemics.

## Adaptation and agronomic recommendations

*Bulala* released for the mid and highland areas of Bale. It is adapted to areas having an altitude ranging from 1700 to 2500 m.a.s.l. with areas

receiving annual rainfall amount ranging from 750 to 1500 mm. Besides, this variety is recommended for production in the areas with similar agro ecologies. Recommended seed is 150kg/ha while fertilizer rate is 41kg/ha and 110kg/ha  $P_2O_5$  and N, respectively. UREA application should be applied in split application- 1/3 at planting and 2/3 at tillering stage to get maximum yield and quality. Planting date is in mid-June to early September.

Table 1. Agronomic and Morphological Descriptors of *Bulala* variety

|   |                                      |                                                         |
|---|--------------------------------------|---------------------------------------------------------|
| 1 | Variety                              | <i>Bulala</i>                                           |
| 2 | Agronomic & morphological characters |                                                         |
|   | 2.1.Adaption area                    | Mid and high lands of Bale                              |
|   | Altitude( ma.s.l)                    | 2000-2500                                               |
|   | Rain fall (mm)                       | 750-1500                                                |
|   | 2.2. Seed rate (kg/ha)               | 150                                                     |
|   | 2.3.Spacing (cm)                     | 0.2 between rows                                        |
|   | 2.4.Planting date                    | Mid-June to early September                             |
|   | 2.5. Fertilizer rate (kg/ha)         | $P_2O_5=69$<br>N=41                                     |
|   | 2.6. Days to flowering               | 68                                                      |
|   | 2.7. Days to maturity                | 136                                                     |
|   | 2.8.Plant height (cm)                | 86                                                      |
|   | 2.9. 1000 seed weight (g)            | 45.1                                                    |
|   | 2.10. Seed color (g)                 | Amber                                                   |
|   | 2.11. Growth habit                   | Erect                                                   |
|   | 2.12. Crop pest reaction             | Resistance to diseases & tolerance to major wheat pests |
|   | 2.14.Proten content (%)              | 13.3                                                    |
|   | 2.15.Gluten (%)                      | 30.3                                                    |
|   | 2.16. Hectoliter weight (kg/L)       | 83.0                                                    |
|   | 2.14.Yield (Qt/ha)                   |                                                         |
|   | Research field                       | 48.2-55.3                                               |
|   | Farmer's field                       | 39.5-76.3                                               |
| 3 | Year of release                      | 2017                                                    |
| 4 | Breeder seed maintainer              | Sinana Agricultural Research Center research center     |

Table 2. Mean grain yield, agronomic performance and disease reactions of 20 durum wheat genotypes tested in durum wheat regional variety trial combined over four locations for years 2012 and 2013

| SN           | Code of genotypes              | Agronomic and disease data |                 |                 |                  |             |                 |             |            |            |
|--------------|--------------------------------|----------------------------|-----------------|-----------------|------------------|-------------|-----------------|-------------|------------|------------|
|              |                                | DH <sup>†</sup>            | DM <sup>†</sup> | PH <sup>†</sup> | TKW <sup>†</sup> | TW          | GY <sup>†</sup> | SR          | YR         | LR         |
| 1            | DW ICARDA -01                  | 71                         | 136             | 85.6            | 43.1             | 83.6        | 4704.9          | 15s         | 5ms        | 5ms        |
| 2            | DW ICARDA -02                  | 70                         | 136             | 85.0            | 40.0             | 83.1        | 4631.8          | 10ms        | 0          | 15s        |
| 3            | DW ICARDA -03                  | 69                         | 134             | 87.7            | 43.5             | 83.7        | 5269.2          | 15ms        | trmr       | 10ms       |
| 4            | DW ICARDA -04                  | 70                         | 136             | 87.2            | 44.2             | 83.7        | 5131.6          | 15s         | 5mr        | 5ms        |
| 5            | DW ICARDA -05                  | 74                         | 137             | 82.2            | 44.8             | 81.8        | 5330.8          | 5ms         | 0          | 5ms        |
| 6            | DW ICARDA -06                  | 73                         | 136             | 81.7            | 43.8             | 82.5        | 4887.8          | 10ms        | 0          | 10ms       |
| <b>Durum</b> |                                |                            |                 |                 |                  |             |                 |             |            |            |
| 7            | <b>ICARDA/EthiopiaPDYT-322</b> | <b>68</b>                  | <b>136</b>      | <b>86.6</b>     | <b>44.4</b>      | <b>82.8</b> | <b>5641.5</b>   | <b>10ms</b> | <b>5mr</b> | <b>5ms</b> |
| 8            | DW ICARDA -08                  | 67                         | 134             | 86.6            | 43.6             | 83.5        | 4613.0          | 5ms         | 0          | 5ms        |
| 9            | DW ICARDA -09                  | 68                         | 135             | 78.8            | 46.2             | 81.5        | 4575.2          | 10ms        | trmr       | 10ms       |
| 10           | DW ICARDA -10                  | 67                         | 137             | 87.4            | 47.7             | 82.4        | 4624.1          | 5ms         | 5mr        | 5ms        |
| 11           | Durum ICARDA/Ethiopia SR.R-6   | 68                         | 133             | 81.4            | 40.8             | 83.1        | 5391.3          | 10mr        | 5mr        | 10ms       |
| 12           | DW ICARDA -12                  | 67                         | 135             | 86.7            | 47.1             | 82.6        | 4651.3          | 5ms         | trmr       | 5ms        |
| 13           | DW ICARDA -13                  | 66                         | 134             | 86.8            | 50.9             | 81.9        | 4721.5          | 5ms         | trmr       | 5ms        |
| 14           | DW ICARDA -14                  | 67                         | 137             | 88.0            | 50.4             | 83.3        | 4660.5          | 10ms        | 0          | 10ms       |
| 15           | DW ICARDA -15                  | 68                         | 135             | 82.8            | 47.9             | 83.0        | 4641.1          | 10ms        | 5mr        | 10ms       |
| 16           | DW ICARDA -16                  | 67                         | 135             | 88.2            | 48.3             | 83.2        | 4384.5          | 5ms         | 5mr        | 10ms       |
| 17           | DW ICARDA -17                  | 67                         | 134             | 86.6            | 44.1             | 82.9        | 4622.5          | 5ms         | 0          | 10ms       |
| 18           | Tate                           | 70                         | 136             | 87.0            | 44.4             | 83.2        | 5278.7          | 15s         | trmr       | 10ms       |
| 19           | Toltu (standard check)         | 69                         | 133             | 79.5            | 40.5             | 83.2        | 4995.6          | 10ms        | 5mr        | 5ms        |
| 20           | Engize (Local check)           | 67                         | 135             | 110.7           | 44.3             | 82.7        | 4609.7          | 10ms        | trms       | 15s        |
| Mean         |                                | 69                         | 135             | 86.3            | 45.0             | 82.9        | 4868.3          |             |            |            |
| CV (%)       |                                | 1.9                        | 1.6             | 5.3             | 5.3              | 3.0         | 12.9            |             |            |            |
| SE           |                                | 0.26                       | 0.43            | 0.94            | 0.48             | 0.51        | 127.72          |             |            |            |
| LSD (5%)     |                                | 0.73                       | 1.21            | 2.62            | 1.34             | ns          | 355.39          |             |            |            |

**Note:** DH= days to heading, DM= days to maturity, PH= plant height (cm), TKW= thousand kernel weight (gm), TW= test weight (kg/hl), GY= grain yield (kg/ha), SR= stem rust (%), YR= yellow rust (%), LR= leaf rust (%), S= Susceptible, ms= moderately susceptible, mr= Moderately resistant, TR: Trace, Trms: Trace with moderately susceptible, Trmr: Trace with moderately resistant, R: resistant, CV(%): Coefficient of variations, SE: standard error of the mean, LSD: Least significant differences

Table 3. Mean agronomic performance, disease reactions and quality parameters of the *Bulala* along with checks evaluated in variety verification trial in year (2016)

| Genotypes                                      | Agronomic, disease and quality data |     |     |    |      |      |      |      |      |            | M %  | Protein % |
|------------------------------------------------|-------------------------------------|-----|-----|----|------|------|------|------|------|------------|------|-----------|
|                                                | DH                                  | DM  | PH  | GY | TKW  | TW   | SR   | YR   | LR   | Gluten (%) |      |           |
| <i>Bulala</i> (DurumIC ARDA/Ethiopia PDYT-322) | 68                                  | 136 | 86  | 56 | 45.1 | 83.9 | 5mr  | 5mr  | Trms | 30.3       | 10.4 | 13.3      |
| Dire                                           | 66                                  | 134 | 76  | 49 | 33.3 | 83.8 | 10ms | 10ms | Trms | 24.9       | 9.7  | 11.7      |
| Durum                                          | 68                                  | 133 | 82  | 52 | 47.8 | 83.7 | 10s  | 10s  | 10s  | 28.1       | 9.9  | 12.8      |
| ICARDA/Ethiopia SR.R-Ingilize                  | 67                                  | 136 | 111 | 46 | 43.3 | 82.9 | 10s  | 5ms  | 15s  | 31.16      | 9.5  | 13.1      |

**Note:** DH= days for heading, DM= days to maturity, PH: plant height (cm), TKW: thousand kernel weight (gm), TW: test weight (kg/hl), GY: grain yield (kg/ha), SR: stem rust (%), YR: yellow rust (%), LR: leaf rust (%), M%: Moisture percentage, S: Susceptible, MS: moderately susceptible, MR: Moderately resistant, TR=, trace, Trms: Trace with moderately susceptible

## Conclusion

*Bulala* is officially released in year 2017 for durum wheat growing areas of Bale highlands for its high grain yield, disease resistance, uniformity, good nutritional quality traits (protein, gluten and test weight), earliness, higher thousand kernel weight and higher biomass than the checks. It is highly preferred by consumers.

## Acknowledgements

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# **Ethiopian Journal of Crop Science (EJCS)**

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