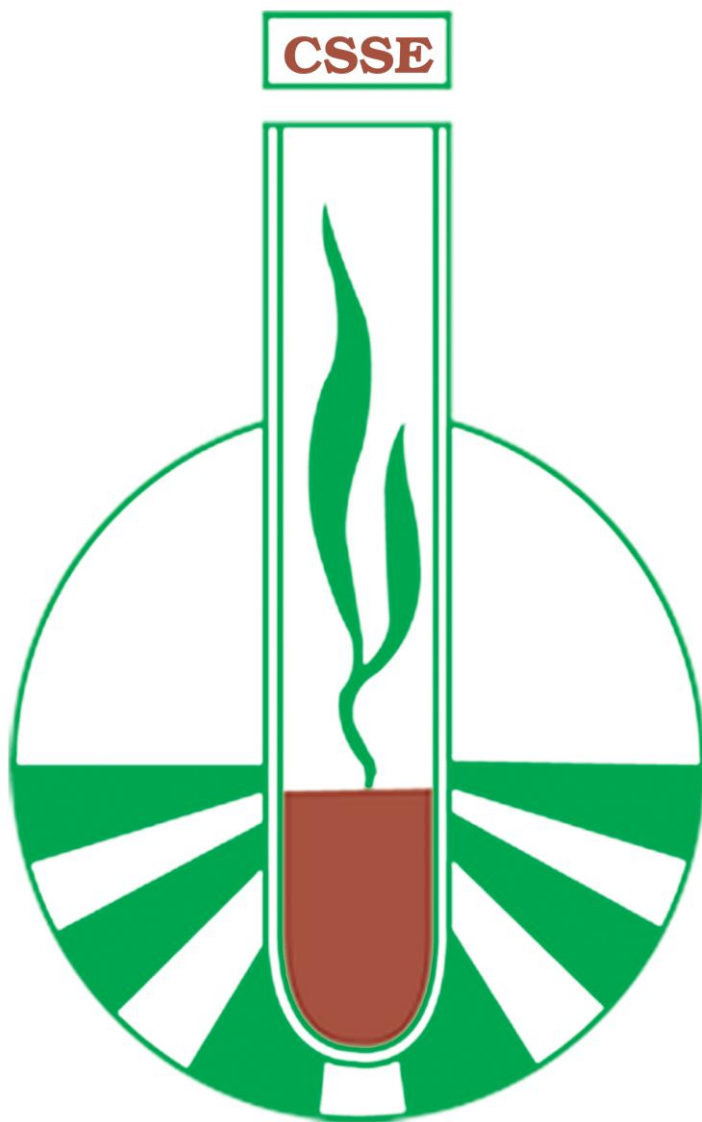


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Genetic Consequences of Failure to Control Outcrossing in Breeding Often Cross-Pollinated Pulse and Oil Crops

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Abstract

Crops with a maximum of 5% natural out-crossing are considered as self-pollinated while those with more than 50% as cross-pollinated. Those with rates of cross-pollination lying between the two, are considered as often cross-pollinated. The economically important often cross-pollinated pulse and oil crops in Ethiopia include: faba bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), sesame (*Sesamum indicum*), Ethiopian mustard (*Brassica carinata*), rapeseed (*B. napus*) and safflower (*Carthamus tinctorius*). In addition to the innate mode of reproduction imparted in a particular crop species or variety, the rate of outcrossing taking place due to external factors favouring cross-pollination, dictate breeders to choose appropriate methods not only in the process of developing new varieties, but also to maintain and perpetuate seeds of improved varieties. For breeders to change the genetic makeup of often cross-pollinated crops towards their interest, they must have a full control over the rate of cross-pollination occurring with mediation of external factors such insect pollinators, wind, birds, water and other agents as is always conscientiously done with cross-pollinated crops. They also need to effectively control pollination to keep released varieties true-to-type during maintenance and multiplication of early generation seed. In Ethiopia, the great majority of often cross-pollinated pulse and oilseed crops are treated, hitherto, as if they were self-pollinators, in the processes of developing elite materials, maintenance breeding and early-generation seed multiplication without diligently controlling free-flow of pollen from unintended sources from the neighbouring plots. As a consequence, effectiveness and efficiency of breeding methods would inevitably get compromised at early stage of the breeding process and varieties lose their genetic identities and thus their potency thereof soon after their release and before they get into the realm of wide scale production. In this review paper, an attempt is made to bring to light the undesirable genetic consequences resulting from failing to control outcrossing in the processes of variety development, maintenance breeding and early generation seed multiplication in often cross-pollinated pulse and oilseed crops in Ethiopia, accompanied with some suggestive recommendations that breeders may consider for possible corrective actions.

Keywords: Cross-pollinated crops, maintenance breeding, often-cross-pollinated crops, outcrossing rate, variety development

Introduction

Plant breeding may be defined in slightly different ways by different scholars, but basically with the same meaning. While some defined it as the science and art of changing genetic makeups of crops to the interest of human (Allard, 1960; Singh, 2004); others defined it as “applied crop genetics” (Sánchez-Monge, 1993). The renowned Russian botanist, geneticist, plant geographer and germplasm curator, Nikolai, Ivanovic Vavilov, noted for his theory on the origin of cultivated plants and the law of homologous series of inherited variation, succinctly defined crop breeding as “crop evolution directed by man” (Vavilov, 1951). Even with the advent of cutting-edge technologies and techniques, such as those in biotechnology, being applied to *de-novo* develop crop varieties, the definition of plant breeding is still maintained to be purposeful or directional alteration of genetic makeups of plants to develop superior crop varieties to the services of human. Whichever way it may be defined, plant breeding involves genetic adjustments of crops to result in the production of new, improved and desirable varieties for human uses (Borém, *et al.* 2002). The new varieties developed as such may have higher yield, improved quality, increased resistance to pests or tolerance to environmental stress factors, or be agronomically more desirable. Ideally, the new varieties

will have combinations of most, if not all, of the desirable attributes which make them significantly better than the varieties already at hand.

Sexually-reproducing crops are broadly categorized as self-pollinators and cross-pollinators. Some others, with intermediate mode of pollination, where cross-pollination exceeds 5% and may reach up to or more than 50%, are considered as often cross-pollinated or partially cross-pollinated or partially-allogamous crops (Githiri *et al.*, 1991; Becker *et al.*, 1992; Keneni *et al.*, 2006; Nabloussi *et al.*, 2013; Gebremichael, 2017). The notion that a plant is capable of self-pollination is different from the essence that crops would be defined as self-pollinators; since the ability to self-pollinate has little to do with the amount of cross-pollination that can occur naturally. This is particularly and practically very relevant with often cross-pollinated crops because insects have an enormous impact on the rate of cross-pollination that can take place in these crops. For example, in crops like faba bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), Ethiopian mustard (*Brassica carinata*), rapeseed (*B. napus*), safflower (*Carthamus tinctorius*) and sesame (*Sesamum indicum*), which are considered as often cross-pollinated crops as the rate of outcrossing in each of them reaches pretty high (Gebremichael, 2017; Downey and Roebbelen, 1989; Githiri *et al.*, 1991; Becker *et al.*, 1992; Keneni *et al.*, 2006; Nabloussi *et al.*, 2013; Teklewold *et al.*, 2013). Since

these crops have intermediate mode of reproduction between strictly cross- and self-pollinated crops, it is possible that they can harbour alleles with undesirable effects concealed in heterozygous state, which may not be noticed during early generations. But at the same time, since the rate of self-pollination occurring in varieties of these crops is so high, the alleles with deleterious effects harboured by their heterozygous state would soon be unravelled following subsequent segregation. These alleles, since they become homozygous upon selfing, would soon inflict their pernicious effects in a full blow. In addition to the innate mode of reproduction imparted in a particular crop species or variety, the rate of outcrossing taking place due to external factors favouring cross-pollination, dictate not only the way breeders should manage their breeding materials at different levels of the breeding scheme, but also the methods they should follow to maintain and perpetuate seeds of the improved varieties they release.

Among the challenges that crop breeders in Ethiopia commonly face with often cross-pollinated crops is the failure to have a full control over the rate of outcrossing taking place in these crops. Most crop breeders in Ethiopia in particular treat often cross-pollinated crops the same way as they treat self-pollinated crops and thus employ the same breeding procedures used for the latter (authors' personal observation). Such a leniency in controlling cross-pollination, as

opposed to the case breeders strictly do with self- or cross-pollinated crops, entails a host of predicaments in their pursuit of shaping genetic architectures of the crops to fit their ultimate desires.

It is, therefore, intended in this review paper to bring into the attention of crop breeders the urgency and need for reconsidering the current schemes being employed to develop, maintain and multiply improved varieties of pulse and oilseed crops generally designated as often cross-pollinated. The purpose of this review is not though to come up with an altogether new breeding methods, for developing varieties, maintenance breeding or early generation seed multiplication for often cross-pollinated pulse and oilseed crops, that are different from the principal methods being used for other crops. The aim is rather to elucidate both practical and theoretical flaws tinted in the breeding techniques and approaches treating often cross-pollinated pulse and oilseed crops in Ethiopia as if they were self-pollinated.

Although the subject of discussion is pertinent to the entire band of crops recognized as often cross-pollinated, particular focus is given to faba bean, pigeon pea, Ethiopian mustard, rapeseed, safflower and sesame where cross-pollination that could occur in these crops either naturally or through mediations of a wide diversity of insect pollinators is condoned. While the overall objective of this review is

to bring to light the genetic consequences of failing to control cross-pollination in breeding and maintenance of often cross-pollinated crops, the specific objectives are to: (1) elucidate the drawbacks of the breeding methods being employed in Ethiopia to develop, maintain and multiply improved varieties of often cross-pollinated pulse and oil crops, particularly faba bean, pigeon pea, Ethiopian mustard, rapeseed, safflower and sesame; (2) forward some suggestive recommendations that would assist breeders to conscientiously limit free-flow of pollen from unwanted or untargeted plants onto the breeding materials; and (3) provide guidance to the respective breeders, engaged in genetic improvement of these crops to warrant their success by increasing the effectiveness and efficiency of breeding methods.

Importance of often cross-pollinated crops in Ethiopia

Often cross-pollinated crops in Ethiopia comprises a large number of economically important crops such as sorghum (*Sorghum bicolor*), cotton (*Gossypium hirsutum*), faba bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), Ethiopian or Abyssinian (as it stands in some literature) mustard (*Brassica carinata*), rapeseed (*B. napus*), sesame (*Sesamum indicum*), and safflower (*Carthamus tinctorius*). Except for pigeon pea and rapeseed, these crops have been produced in

Ethiopia since antiquity and the country is considered to be either the primary or secondary Vavilovian center of origin or diversity (Vavilov, 1951; Frankel, 1973; Harlan, 1973; Westphal, 1974; Engels *et al.*, 1991).

Close to three million hectares of land is currently cultivated under the above-indicated often cross-pollinated crops, except cotton, in Ethiopia, from which over six million tons of yield is harvested, significantly contributing to the livelihood of more than ten million farming-households (CSA, 2018). Most of the crops are produced by smallholder farmers under rainfed and marginal growing situations for different purposes, including: food and feed (as sources of carbohydrate, protein and fat), household income, national export earnings and industrial raw materials. With respect to their importance, sorghum, for instance, is the staple crop in most parts of the country where the condition is too dry for the rest of major cereals. From the export of sesame alone, Ethiopia, on the average, has been earning over 413 million USD per annum for the last five years (NBE, 2017). Faba bean exports also reached 22 million USD in 2014 (NBE, 2016). In the earlier days, Ethiopia used to annually earn close to 2.3 million USD from the export of cotton (MoARD, 2005), in addition to the amount used to cover domestic needs; which accounted for 83% of the total annual production (Mulat *et al.*, 2004).

Faba bean and pigeon pea, in addition to the aforementioned merits, also play significant ecological roles in soil fertility restoration by fixing atmospheric nitrogen (Adu-Gyamfi *et al.*, 1997; Keneni *et al.*, 2016). They are suitable for rotation with cereals (Adu-Gyamfi *et al.*, 1997; Gorf, 1998), enhance smallholders' savings as they need to use less nitrogen fertilizers (IFPRI, 2010), but yet with better sustainability to their farming system (Gorf, 1998; Bejiga, 2004; Hailemariam and Tsige, 2006; Keneni, 2012). For instance, nitrogen fixation by faba bean was found to have significant spill-over effect to subsequently grown cereals in Ethiopia (Gorf, 1998; Woldekirstos *et al.*, 2010) and elsewhere (Kopke and Nemecek, 2010). Nitrogen fixation by pigeon pea also showed significant positive spill-over effect on subsequently grown cereals (Adu-Gyamfi *et al.*, 1997). The legume crops also serve to break the cycles of crop-specific pathogens of cereals (Malhotra and Singh, 2004; Kirkegaard *et al.*, 2008; Kopke and Nemecek, 2010). Pigeon pea plays a unique role in nutrient recycling by solubilising nutrients in the soil, which otherwise would be unavailable to the follow-on crop (Adjei-Nsiah, 2012).

The significance of Ethiopian mustard and rapeseed, as sources of edible oil in Ethiopia, has been well established (Belayneh and Alemayehu, 1986). Safflower is among the oldest plants used for extraction of edible oil and also dyeing foods, beverages and cloth

as red or orange or yellow dyes containing active substances called carthamin or carthamidin are extracted from dried florets. Recently too, the crop is regaining its importance in the food industry as synthetic coloring substances have become virtually shunned by food processors and consumers alike (Lehto *et al.*, 2017).

Over the past fifty years of agricultural research in Ethiopia, quite a large number of improved varieties of these crops were developed and released along with proper crop management and protection practices. According to the MoANR (2016), the number of varieties released, *hitherto*, stands at 48 for sorghum, 29 for cotton, 32 for faba bean, 26 for sesame, eight for the two oilseed brassicas and two for safflower.

Genetic Structure of Self- and Cross-pollinated Crops

Depending on their mode of reproduction, crop varieties fall in one of the four possible categories of genetic structure determined by considering the population of the varieties at genotypic and phenotypic levels. That is, at genotypic level, individuals of a given variety could be either homozygous or heterozygous; while phenotypically the population of the variety could be either homogeneous or heterogeneous (Acquaah, 2007; Becker, 2011). The four categories of genetic structure of varieties as depicted in Fig. 1 are: (i)

homozygous and homogeneous varieties, (ii) heterozygous and homogeneous varieties, (iii)

homozygous and heterogeneous varieties, and (iv) heterozygous and heterogeneous varieties.

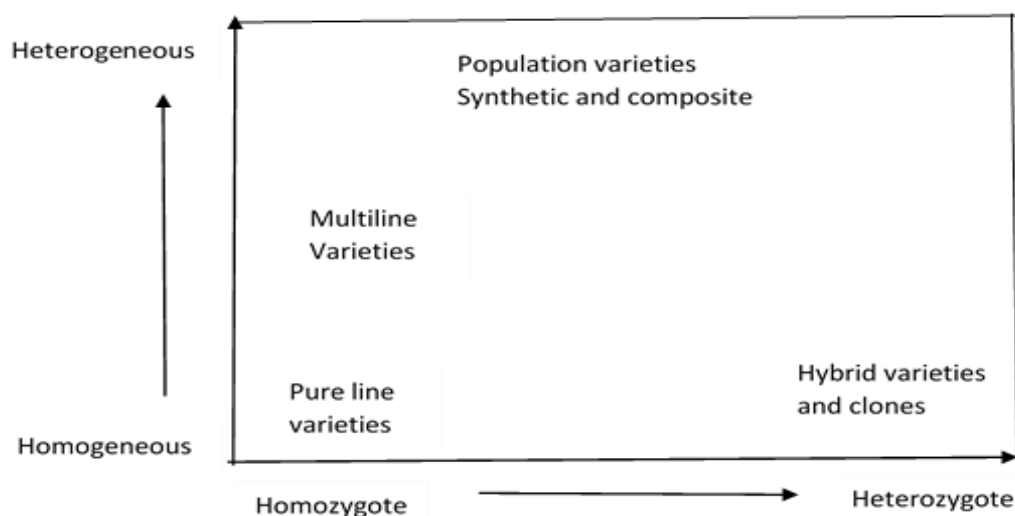


Figure 1. Schematic representation of possible genetic structure of varieties of self- and Cross-pollinated crops (Adapted from Becker, 2011)

Because of such extreme differences of genetic structure of varieties, different breeding methods need to be applied for the different groups as briefly discussed hereunder.

Homozygous and homogeneous varieties: Pure line varieties of self-pollinated crops are derived from a single plant which is genotypically homozygous. Since the whole population of such a variety originates from a homozygous single plant, the variety is expected to exhibit a homogeneous phenotype. Therefore, it is essential that the breeding methods

to be applied should be geared toward producing varieties that are homozygous. Multiline varieties, as could be seen from Fig.1, phenotypically fall somewhere in the middle implying that the individual plants are genotypically homozygous, but are derived from plants of different genetic background. Thus, as single entities multi-line varieties are genotypically homozygous but they are phenotypically to a considerable extent heterogeneous.

Heterozygous and homogeneous varieties: Breeding methods may

need to be applied to specifically result in a variety that is genetically heterozygous and yet is phenotypically homogeneous. Breeding hybrid varieties is a typical method for achieving such a genetic structure of varieties. The heterozygous genetic structure stems from the fact that a hybrid cultivar is the F_1 product of a cross of highly inbred parents. Crossing such pure lines produces highly heterozygous F_1 plants. Because the F_1 is the final product released as a new variety, all plants are uniformly heterozygous and hence homogeneous in appearance. Clones may be very heterozygous but the offspring of a clonal population are homogeneous. Once the genotype has been altered in a desirable way, the changes are fixed as long as propagation is made using clones.

Homozygous and heterogeneous varieties: These are varieties where the individual plants are genotypically homozygous but the individual genotypes constituting the variety are not homogeneous. Typical examples of such a variety are the mixed landraces that are developed and

recognized as farmers' varieties. The extent of heterogeneity in landraces is much broader than is in multiline varieties.

Heterozygous and heterogeneous varieties: These are varieties with heterozygous individual plants but with relatively homogeneous appearance. Typical examples include varieties of cross-pollinating crops developed through synthetic and composite breeding methods. Composite and synthetic varieties, unlike hybrid varieties, allow farmers to reuse their own seed at least for some generations before they need to acquire new stocks of seed.

Often cross-pollinated crops share the genetic features of both self- and cross-pollinated crops and as such combinations of the different genetic structure could coexist in a single variety. Developing superior varieties with the right combination of multi-locus homozygosity along with multi-locus heterozygosity and a gradient of homogeneity all in one makes the breeding programme tiresome and limiting.

Breeding Methods used to Develop Varieties of Often Cross-pollinated Pulse and Oilseed Crops in Ethiopia

The breeding methods used worldwide to develop improved varieties of crops are innumerable based on the mode of reproduction as this is the governing characteristic. Notwithstanding the multitudes of modifications breeders make, there are some universal methods though which are distinct for cross or self-pollinated crops. Therefore, providing first a bird's-eye view of the breeding methods used to improve cross- and self-pollinated crops lays the foundation to understand and criticize the merits or demerits of particular methods used for often cross-pollinated crops as this is the main purpose of this review article.

Overview of breeding methods used with cross- and self-pollinated crops

The core task of a plant breeder is to acquire or create genetic variations and select better types, from out of the variants, in yield and/or quality of usable parts; desirable attributes of cultivation, harvesting, threshing, grading and processing; tolerance to environmental stresses; and resistance against pests. Scholars of both earlier (Allard, 1960) and recent (Bressegello and Coelho, 2013) times sort breeding methods into three categories as breeding based on: selection of

variants naturally occurring within traditional varieties, landraces or collections; controlled mating and selection of plants with recombination of desirable genes; and monitored recombination by selection of specific genes or marker profiles, using molecular tools for tracking within-genome variation.

Breeding methods used with self-pollinated crops: It is a plain truth that the mode of reproduction of crops is the singular factor dictating which breeding methods to employ for developing new varieties. Breeding methods that have long been established successful for self-pollinated species are: mass selection; pure-line selection; hybridization, with the segregating generations handled by the pedigree, bulk, or backcross methods; and development of hybrid varieties (Allard, 1960, 1999; Becker, *et al.*, 1992). Self-pollination increases homozygosity at individual plant level as well as homogeneity at population level. Self-pollinated crops, unlike their cross-pollinated counterparts, do not suffer from the genetic consequences of inbreeding.

Mass selection in self-pollinated crops is starts by collecting seeds from a large number of phenotypically desirable individuals in a population and the next generation is grown from the stock of mixed seed. Mass selection has been used to improve old "landrace" varieties, which have been passed down from generations over long periods. Replacement of

traditional varieties with modern, uniform cultivars leads to genetic erosion of landraces unless the latter are conserved ex-situ or in-situ in a unique form of community-based conservation programs. The consequence of landrace genetic erosion is a decrease in genetic diversity which means genes and alleles will not be available for breeders to develop improved varieties. Cultivars grown by farmers become increasingly genetically homogenous.

An alternative approach that has been practiced for thousands of years is simply to eliminate undesirable types by destroying them in the field. The results are similar whether superior plants are saved (positive mass selection) or inferior plants are eliminated (negative mass selection) as seeds of the better plants become the planting stock for the next generation. A modern refinement of mass selection is to harvest the best plants separately and to grow and compare their progenies so that the poorer progenies would be destroyed and the seeds of superior individuals are harvested. Selection is thus based not solely on the phenotype of the parent plants but also on the performance of their progenies (Jeromela, *et al.*, 2016), which makes the method more effective especially for quantitative characters of low heritability.

A pure line is a progeny of a single, homozygous, self-pollinated plant. Pure-line selection generally involves

three distinct steps: first, numerous phenotypically superior plants are selected from a genetically varying population; then progenies of the selected plants are grown and evaluated subsequently over several generations; and finally, when it becomes difficult to select individual plants based on their phenotype alone, extensive field trials are conducted to determine whether the remaining selections are superior in performance. Any progeny superior to an existing variety would then be released as a new “pure-line” variety. The success of pure-line method, to a greater extent, depends on the existence of genetically diverse landraces or cultivars where genotypes are waiting to be exploited (Osei, *et al.*, 2014). These cultivars are homogeneous and homozygous in genetic structure, a condition attained through a series of self-pollinations. These cultivars are often used as parents in the production of other kinds of cultivars. Pure-line cultivars have a narrow genetic base. They are desired in regions where uniformity of a product has a high premium (Acquaah, 2007).

Planned hybridization followed by a systematic selection of desirable genotypes from the segregating populations has become nowadays the dominant breeding method of self-pollinated species. The hybridization is meant to combine desirable alleles found in two or more different cultivars and develop a superior pure-breeding variety packed with all the genes controlling the desirable traits.

The problem though has always been on handling the enormous numbers of segregating populations resulting from hybridization, although it is a very powerful means of creating variability. We recall the polynomial expansion formula of polygenic inheritance, $(a + b)^n$; where a and b are the parental genotypes differing in “ n ” number of genes.

If, for example, a hypothetical cross between two parental varieties of field pea (a highly self-pollinated species) differing in only 6 genes, assuming that there are only two alleles segregating at each locus, the minimum number of plants required to represent all possible segregating genotypes expected at the second generation of selfing (F_2) from the crossing is 64, as is illustrated by the following binomial expansion:

$$(a + b)^6 = a^6 + 6a^5b + 15a^4b^2 + 20a^3b^3 + 15a^2b^4 + 6ab^5 + b^6$$

It can be deduced from the above model that if the number of genes is to increase, say, to ten, maintaining the same assumption of having only two alleles at a locus, then the minimum number of individual F_2 plants that need to be generated, to represent all possible recombinants from the crossing, rises to 1024. Even at such rapidly increasing number of F_2 plants that need to be raised, the chance of recovering genotypes on the end sides of the distribution (i.e., genotypes with the coefficient of “6” on the right and left sides in the above equation) is always much lower than those of genotypes around the middle of the distribution (i.e., with coefficients of 15 and 20). This again implies that unless otherwise the theoretical minimum number of F_2 segregants is substantially increased, the chance of recovering genotypes on the extreme sides of the distribution would practically be too little to rely on.

Furthermore, the number of genes assumed in the above example to illustrate the enormity of segregating populations that the breeder has to handle is in fact too little compared to the number of genes in, for example, Ethiopian mustard, involved in most quantitative traits like protein, oil, or glucosinolate contents which are of prime importance for the oilseed breeding programs in Ethiopia (Alemayehu and Becker, 2005). So, if the full spectrum of genotypic variation resulting from hybridization of parents differing in many genes is to be captured, the sheer number is going to be daunting in terms of the time and other resources that breeders need to invest in order to properly manage the segregating populations and increase their chances of identifying genotypes of interest. The problem will even get further complicated if one considers possible linkages and epistasis occurring

among alleles controlling different traits.

Thus, breeders need to choose techniques that would enable them to handle and manage such enormous number of segregating populations in the best way possible to optimize resource utilization but, without jeopardizing genetic gains. There are a number of methods, to choose from or modify to fit specific circumstances without losing sight of the principle, for handling and selecting segregating populations of self-pollinated crops as briefly discussed hereunder.

Pedigree breeding starts with the crossing of two genotypes, each of which has one or more desirable characters but lacking in the other. In the pedigree method, superior types are selected in successive generations, and a record is maintained of parent–progeny relationships (Jeromela *et al.*, 2016). The bulk-population method of breeding differs from the pedigree method primarily in the handling of generations following hybridization. No pedigree is kept with bulk method of advancing segregating generations, but natural selection plays a significant role in eliminating genotypes with poor performances. Single plant selections are made at F_4 or F_5 stage and evaluated in the same way as in the pedigree method of breeding. The chief advantage of the bulk population method is that it allows the breeder to handle very large numbers of individuals with less cost.

With backcross breeding, the F_1 generation is repeatedly crossed back to the recurrent or recipient parent while the non-recurrent or donor parent is used only to introgress new genes, which are deficient in the recipient parent. By repeatedly backcrossing to the recurrent parent it is possible to recover the whole genotype of the recipient parent except for the traits or the genes to be introgressed. It is, therefore, a method very useful to transfer simply inherited genes like, for instance, resistance or quality genes into a commercial variety, which lacks these genes, but is otherwise well adapted and high yielding. Backcross breeding doesn't create new variability but only uses allelic differences already available at a specific locus. In backcross breeding, there is no need for an obligatory yield testing and thus there will be no waste of additional time and money for variety evaluation, since the performance of the recipient genotype is predictable.

Due to restricted genetic makeup, low magnitude of heterosis, low yield, lack of sound male sterility system, tedious hand emasculation and other characteristics associated with self-pollinating crops, which pose difficulties in hybrid seed production at commercial scale, hybrid breeding was limited to only cross-pollinated crops (Whitford, *et al.*, 2013; Kumar, *et al.*, 2014). Recently, however, hybrid breeding has become a commonplace with self-pollinating crops as well; as successful yield

benefits from heterosis are being exploited in wheat and rice where yield improvements from heterosis reaching up to 15 and 16% were realized with commercial varieties of the two crops, respectively (Cheng, *et al.*, 2007; Longin *et al.*, 2012).

Breeding methods used with cross-pollinated crops: Allard (1960) asserts that the most important methods of breeding cross-pollinated species are: mass selection; development of hybrid varieties; and development of synthetic varieties. Since cross-pollinated species are naturally hybrid (heterozygous) for many traits and lose vigour as they become purebred (homozygous), the goal of each of these breeding methods is to preserve or restore heterozygosity.

In mass selection, whether in cross- or self-pollinated crops, a large number of superior plants are selected and harvested in bulk and the seed is used to produce the next generation. Therefore, a variety developed by mass selection is made up of genotypes bulked together without having undergone combining ability test. In hybrid production, on the other hand, three steps are essentially to be followed; i.e., selection of superior plants from a source population; selfing the selected plants for several generations until they become inbred lines, which although differ from each other are pure-breeding and highly uniform; evaluation of inbred-lines for combining ability; and making

crossing between two or more selected inbred lines to develop hybrid variety.

Development of hybrid varieties is meant to produce genotypes which are more vigorous than their parents. The hybrid vigor, or heterosis, can be manifested in many ways, including increased rate of growth, greater uniformity, earlier flowering, increased yield, and other traits of interest. Heterosis of a hybrid is expected to increase, within a limit, with the genetic divergence between its parents and thus, systematic grouping of lines into genetically divergent heterotic pools (comprehensive breeding system) is essential to make maximum use of the heterosis (Melchinger, 1999). Although the vigor of the lines decreases enormously during the inbreeding process, it is restored upon crossing the inbred lines.

The third method, which is development of synthetic varieties, involves intercrossing of a number of genotypes with well-established combining ability of superior performance, with respect to the trait of interest. This would in principle ultimately bring together genotypes that are known to give superior hybrid performance when crossed in all possible combinations.

Overview of breeding methods used to improve often cross-pollinated crops

Depending on the traits of interest to the breeder, different breeding schemes are being used to improve often cross-pollinated crops. The core principle embedded in all the methods though is to choose suitable parents that would most likely result in progenies containing genes of desirable and targeted traits (Jeromela *et al.*, 2016). Unlike self-pollinated crops, the parents chosen need to be selfed for a few generations in often cross-pollinated crops to produce homozygous lines that would serve as parental lines in breeding programmes (Kumar, 2006). Then methodical selection procedures are applied that are both effective and efficient in sifting out recombinants and developing them into full-fledged new varieties (Jeromela *et al.*, 2016).

Often cross-pollinated crops show genetic features of both self- and cross-pollinated crops. On one hand they show low inbreeding depression like self-pollinated crops; and, on the other hand, they possess high heterozygosity in their natural population, and exhibit high heterosis like cross-pollinated crops (Singh and Shekawat, 2018). As the result of these dual features of often cross-pollinated crops, both pure lines of a number of often cross-pollinated crops (MoA, 2017) and hybrids of sorghum have been safely released in Ethiopia (Tadesse, *et al.*, 2010) and elsewhere

(Singh and Shekawat, 2018) for wider production. Hybrid seed production is also simplified in these crops because of the common feature they share with cross-pollinated crops (Singh and Shekawat, 2018).

The dual genetic feature also enables plant breeders to apply breeding methods suitable for both self- and cross-pollinated crops in order to improve often cross-pollinated crops (Singh and Shekawat, 2018). Plant breeders use many established techniques and methods such as population improvement (mass and recurrent selections), pedigree method, half-seed techniques (rapeseed and Ethiopian mustard), backcross breeding, bulk method, single seed descent and hybrid breeding (Downey and Roebbelen, 1989; Jeromela, *et al.*, 2016). Production of hybrids particularly requires development of cytoplasmic male sterility (Wang, *et al.*, 2007; Jeromela, *et al.*, 2016), interspecific breeding which involves generation of haploidy through microspore culture and embryo rescue, protoplast fusion, resynthesis, and advanced biotechnology methodologies (Diederichsen, *et al.*, 2009), such as marker assisted selection (MAS) and genetic engineering.

Diverse conventional methods are being used with often cross-pollinated crops to breed varieties of interest, especially to improve yield, quality and adaptation. In every step of selection, generation advancement is

based on progeny-test. One of the limitations in breeding of often cross-pollinated crops, be it in variety development or evaluation process, is that a wide range of experiments with diversities of objectives, different stages of selection and most importantly with varieties of collections of genotypes are simultaneously tested side-by-side without having any control over interplant dispersal of pollen. Seeds directly obtained from such trials are also used to constitute subsequent trials and initial seeds for the ultimate variety. This has emanated from the notion that breeders in Ethiopia, very commonly, treat often cross-pollinated pulses and oilseeds the same way as they treat self-pollinated crops. Consequently, they usually employ the same breeding procedures used for the latter without due regard to the amount

of cross-pollination taking place in these crops. Nevertheless, mostly as a result of relentless visits by a host of insects particularly honeybees, carpenter bees and a variety of other foraging insects (Atmowidi, *et al.*, 2007; Marzinzig, *et al.*, 2018), a significant amount of out crossing occurs in these crops (Table 1). Such a leniency in controlling cross-pollination, as opposed to the case breeders strictly do with cross-pollinated crops, entails quite a lot of predicaments in pursuit of shaping the genetic architecture of the crops to fit their ultimate desires. Direct use of seed from the previous generation in order to advance next generations would, each time, result in genetic contamination and loss of identity in the breeding materials.

Table 1 The rate of outcrossing occurring in the major often cross-pollinated pulse and oilseed crops grown in Ethiopia

Crop	Rate of Outcrossing (%)	Reference
Pigeon pea	13	Githiri <i>et al.</i> , 1991
Faba bean	30	Keneni <i>et al.</i> , 2006; Adi, 2007
Safflower	27	Nabloussi <i>et al.</i> , 2013
Ethiopian mustard and rapeseed	30-47	Downey and Roebbelen, 1989; Becker <i>et al.</i> , 1992; Teklewold <i>et al.</i> , 2013
Sesame	45	Alemberhe and Gebremeskel, 2016; Gebremichael, 2017

Genetic Consequences of Outcrossing in Often Cross-pollinated Crops

The genetic architecture of partially allogamous or often cross-pollinated crops is intermediate between self- and cross-pollinated species. The rate of cross-pollination occurring in these

crops varies from a low of around five percent to anything up to 50% or more depending on the biology of the crop and affinity of insects which affect the rate of outcrossing to a great extent (Suso, *et al.*, 2008). Plant breeders dealing with this league of crops should, therefore, use quite a range of

modifications of the principal methods originally developed for either self- or cross-pollinated species to suit their specific conditions.

The amount of cross-pollination, i.e. the transfer of pollen from the anther of one flower to the stigma of another flower on a different individual plant of same or different species, in different often cross-pollinated crops may reach well over 50% as indicated above. The boost in production and productivity gained from improved and released crop varieties, whatever their mode of reproduction may be, is by and large attributed to the extent of improvements made in the genetic makeups of the varieties. Despite this core essence of altering genetic architecture to be assured by properly controlling pollination, as the basis of developing improved crop varieties, often cross-pollinated crops are liable to frequent loss of identity in the processes of selection and evaluation of breeding materials in nurseries; varietal testing, maintenance breeding and initial seed multiplication for the mere fact that they are treated like self-pollinating crops.

In the process of progeny selection, subsequent evaluation, breeder seed maintenance and multiplication, the genotypes grown side-by-side exchange genes and ultimately lose their original genetic identities. In the presence of pollinators, inter-crossing is inevitable among genotypes of different genetic background, and thus the identity of genotypes of interest

would be lost after only few generations because of unwanted gene-flow and recombination. In terms of undesirable genetic consequences of treating often cross-pollinated pulse and oilseed crops as if they were self-pollinated, the following logical critiques are narrated on the breeding methods:

Critiques on fixation of desirable genotypes

The breeding programs on pulses and oilseeds have contributed a lot to the betterment of the sector by developing and promoting improved varieties that have better agronomic and economic merits. Notwithstanding this, however, the breeding procedures employed and achievements secured are never immune of methodical gaffes and technical limitations.

Thus, if the seed from the same progenies grown under such complex conditions to be advanced, is directly used to constitute the next generation, as is done for highly self-pollinated crops, it is not possible to dictate and direct varietal evolution for traits of interest towards the intended targets. This is because of free-flow of unwanted genes among genotypes of different genetic background when grown side-by-side (Sharma, 2001). It is also difficult to keep released varieties true-to-type during maintenance breeding and at initial increase of early generation seeds unless pollination is effectively and efficiently controlled (Keneni, *et al.*, 2001; Singh, 2004).

The theoretical description of what commonly happens if pollination is not well controlled in often cross-pollinated crop varieties with varying amounts of inter-crossing during variety development, variety testing, breeder seed maintenance and initial increase of early generation seeds was given by Witcombe (1982). The author employed a hypothetical example for faba bean where two genotypes differing in a single locus so that the initial difference in gene frequency would be 1. It could be discerned from Figure 2 that only after three generations and 50% possible intercrossing, the difference in gene frequency at this locus is reduced from

1 to 0.1; and in the same case when intercrossing reaches 50%, it would take only six generations for two such genotypes to become completely identical. It should also be noted that it takes more than six years to get to this level even in the breeding nurseries where different crossing/backcrossing and selection schemes are followed. The corresponding period may be doubled when inter-crossing is 25% but that is still not long enough from plant breeding perspective. Note also that, if properly handled, there are varieties of often cross-pollinated crops like faba bean which served well over 20 years after their release (MoA and ATA, 2017).

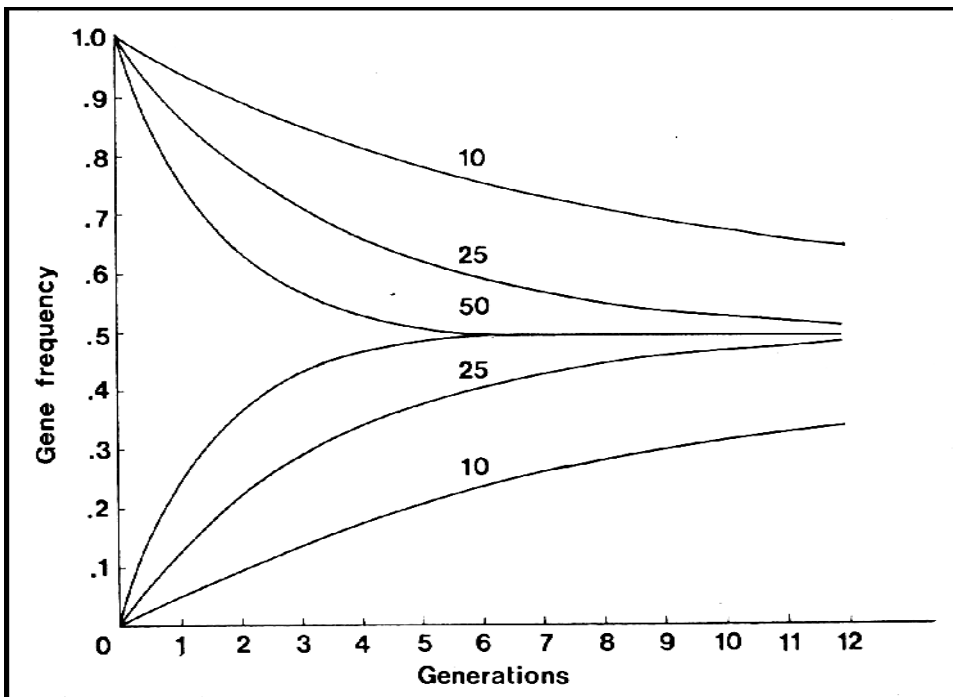


Figure 2. Illustration of the rate at which two genotypes, initially differing for a gene, become identical at 50%, 25% and 10% inter-crossing (Source: Witcombe, 1982)

Critiques on effectiveness and efficiency of the breeding methods

Genotypes of crops with the best combination of economically as well as agronomically desirable characteristics are of rare occurrence in nature and their artificial constitution, through plant breeding, needs meticulous application of lengthy technical procedures, dedication, endurance and often backbreaking physical indulgence. Acclaimed trailblazers of plant breeding (Allard, 1960; Schnell, 1982) and succeeding scholars (Moreno-González and Cubero, 1993; Gepts, 2002) edify that a plant breeding program is a cyclic process where each cycle consists of three phases: generating variation and eliminating undesirable germplasm material through selection and testing to identify superior recombinants which is done with different not only conventional methods but also using modern techniques such as marker-assisted selection, introgression of quantitative trait loci (QTL), or use of high-throughput phenotyping platforms; and release, distribution and adoption of new cultivars.

With increasing complexity of breeding programs, there are important technical as well as economic implications that need to be considered for breeding programs to successfully thrive. The successes are measured by the extent of effectiveness of the breeding programs in terms of

selection gain or response to selection, adoption of varieties, and the benefit/cost ratio of the new variety (Allard, 1960; Ceccarelli, 2015). Response to selection is mathematically defined as: $R = Sh^2$ (Falconer, 1981); where S is the selection differential (i.e., the difference between the mean of the selected individuals and the mean of the whole population), and h^2 is heritability of the target trait. The S is determined by the intensity of selection (i), which, in turn, depends on the percentage of individuals selected and is equal to S/σ_p , where σ_p is the square root of the phenotypic variance, modifying the whole equation to be expressed as $R = i h^2 \sigma_p$.

Eberhart (1970), long before Ceccarelli's assertion of increasing efficiencies of breeding methods, as cited above, introduced the number of seasons per cycle (γ) into the formula, so that it becomes in a simplified form, $R = (i h^2 \sigma_p) / \gamma$. The equation serves to distil theory down to parameters that a breeding program can manipulate to increase the efficiency of its breeding methods. So, a particular selection method which reduces the number of seasons required to come up with genetically stabilized genotypes of desire without compromising genetic gain increases breeding efficiency.

If pollination in often cross-pollinated crops is not well controlled, there would be random mating between unintended parents and their progenies instead of the intended selective

mating of parents with high breeding value in a closed population. Selection from out of such genetically polluted source materials will not obviously be successful to achieve the kinds of varieties desired. Therefore, whatever complex and sophisticated the breeding procedures we may follow, the outcrossing taking place in often cross-pollinated crops would reduce the response to selection per season ($R = (i h^2 \sigma_p) / \gamma$) relative to what could have been achieved through selective mating of parents with high breeding value in a closed population (Cobb *et.al.* 2019). In such a situation where the source materials are being genetically contaminated with alien pollen while the breeding process is going on, accurate prediction of the expected genetic gain from selection as expected would be clearly impractical.

Critiques on maintenance breeding and early-generation seed multiplication

Breeder seed is the earliest generation seed, and any genetic contamination at this stage will be difficult to correct at a later stage. Loosing quality of breeder seed due to genetic contamination means that the starting material for all hierarchal seed classes (i.e., pre-basic, basic and certified seeds) would be lost. The danger of treating often cross-pollinated crops as self-pollinators jeopardises the process not only in the breeding nurseries and yield trials but also during breeder seed maintenance and initial increase

of early generation seeds. For instance, the variety of sesame known as *Adi* (means white in Oromifa), seeds of which were pure white at the time of its release, has now become a mixture of black and white. Similarly, the seed-color of *Yellow Dodola*, a variety of Ethiopian mustard which was pure yellow at its release (Belayneh and Alemayehu, 1986), has now become a mixture of yellow and brown. It is also to be remembered that pigeon pea varieties were once rejected for release by the evaluating team at verification level because of lack of genetic uniformity. The lack of their uniformity might have resulted from contamination of unwanted genes harbored by the genotypes in the process of selection and evaluation, while they were in the breeding nurseries and through stages of yield trials. On the other hand, experimental evidences from faba bean (Keneni, unpublished) showed that proper maintenance breeding of even old varieties with broader genetic base could result in restoration of originality of the varieties. Furthermore, new traits such as resistance to diseases or races that are altogether new or were not so important by the time when the varieties were released could also be incorporated.

The discussion laid out above, however, should not lead to erroneous conclusions about the benefits that pollinating insects impart in often cross-pollinated crops. Insect pollinators significantly enhance the yield potential of these crops (Alemberhe

and Gebremeskel, 2016) as they improve fertilization within a population and thus seed setting and yield thereof. Insect simulated hand-tripping of flowers in faba bean genotypes, for instance, was found to improve seed setting by 30%, albeit different genotypes responded differently based on their auto-fertility levels (Keneni *et al.*, 2006). Results of a two-year field study designed to assess the effects of supplemental honey bees on faba bean productivity showed a yield advantage of over 22% in Ethiopia (Adi, 2007) and 25%-49% elsewhere depending on the activity of pollinating insects, genotype (autofertile or non-autofertile) and the environment (Moreno-González and Cubero, 1993; Somerville, 2002; Musallam, 2004). The reason for yield improvement, at least in part, is associated not only with direct improvement of fertilization but also with the releases of the stigma and style from the keel petals and rupturing of the waxy stigmatic surface that normally hinders pollination by insects (Witcombe, 1982).

Redirecting the Breeding Scheme being used for Often Cross-Pollinated Pulse and Oilseed Crops in Ethiopia

As one can deduct from the discussions above, the purpose of this review is to elucidate both practical and theoretical flaws tinted in the breeding techniques and approaches treating often cross-

pollinated pulse and oilseed crops in Ethiopia as if they were self-pollinated. The purpose of this chapter is to uncover the genetic consequences of failing to control free-flow of non-target genes via outcrossing from unknown pollen-sources that is occurring in often cross-pollinated pulse and oilseed crops and thereby to invoke the need for revisiting the breeding techniques being employed for variety development, maintenance breeding and seed production in these crops.

Pollination control in varietal development

Since significant level of outcrossing between varieties and strains of different genotypic constitution is common in often cross-pollinated pulse and oilseed crops, effective pollination control in breeding nurseries is essential to avoid undesirable recombination of genes arising from uncontrolled outcrossing. The effectiveness of a breeding scheme first and foremost depends on the choice of suitable parents and application of most appropriate techniques for efficiently and accurately selecting and handling the progeny. One distinguishing feature of often cross-pollinated crops, unlike self-pollinated ones, may be the need for the parents chosen for breeding to pass through more generation of selfing in order to select the desirable homozygous parental lines (Kumar, 2006).

There are a number of different methods of excluding pollinator insects in the breeding process including hand emasculation followed by pollination and bagging of individual plants or individual flowers. Insect proof pollination cages could also be constructed by covering wooden or metal frames with screen meshes or spun polyester material. Experiences from faba bean breeding program at Holetta Agricultural Research Center of Ethiopia corroborate that, it is possible to avoid uncontrolled inter-crossing between entries by using insect-proof breeding cages (18 m wide × 30 m long) adopted from ICARDA (Figure 3), which is found to be effective in

excluding pollinating-insects (Jarso *et al.*, 2008). The insect-proof breeding cages are particularly useful at the initial stages of breeding where genetic materials need to be repeatedly selected and advanced in nurseries until they attain genetic homogeneity. It is, therefore, imperative that the breeding programs dealing with the other often cross-pollinated pulses and the entire oilseeds with similar mode of pollination, particularly the two oilseed brassica species, may follow suit in using this innovative technique of curtailing genetic contamination of breeding materials in nurseries due to uncontrolled transfer of foreign pollen often mediated by pollinating insects.



Figure 3. Typical example of insect-proof breeding cages used for faba bean breeding nursery where different filial generations are advanced to next level of progeny (Photo: curtsy of ICARDA¹)

¹ ICARDA (International Center for Arid and Semi-Arid Agriculture, Aleppo, Syria)

Another positive experience from the faba bean breeding program in Ethiopia that is worth to consider by breeders of other often cross-pollinated crops is the “remnant seed technique” where at later stages of nursery-level evaluations, just before genotypes are taken to multi-environment yield trials, half of the seed is kept safely as a reserve backup (preferably in a cold store in hot areas) while the remaining half is used to conduct the field trials. The same seed harvested from the field maybe recycled to grow the next generation of yield trial without posing control over cross-pollination. At the end of the yield trials, however, the remnant backup seed in the stock is retrieved for growing the candidate variety to be verified and evaluated by the relevant body before it is officially released and registered. Once the variety is officially released, the same stock of remnant seed is used as a nucleus for multiplication of early-generation seed (i.e., breeder and pre-basic seed). Experiences from elsewhere (Downey and Roebbelen, 1989) also shows that the remnant seed technique is appropriate for breeding rapeseed, which is an often cross-pollinated oilseed crop.

Pollination control in maintenance breeding of improved varieties

Genetic deterioration of often cross-pollinated crop varieties, once entering into production cycles, can be caused

during generation advancement by natural crossing while the variety is still in the hands of plant breeders; most importantly by the techniques and methods used for maintenance breeding. Since the rate of outcrossing occurring in self-pollinated crops is practically negligible, the genetic constitutions of such varieties remain fairly stable long after the varieties have been released. Thus, both breeders and farmers may repeatedly grow seeds from the same source of generations over years without losing genetic trueness of the varieties as long as mechanical mixtures are avoided. Whereas in often cross-pollinated pulse and oilseed crops like faba bean, pigeon pea, Ethiopian mustard, rapeseed and sesame, where a considerable amount of outcrossing takes place, improved varieties can easily get contaminated after few years of release unless precautionary measures are taken to exclude foreign pollens from unknown sources that could cause genetic deterioration of the varieties. It is, therefore, utterly important to periodically get rid of plants with such genotypes, no matter how small their number may be in the population, or if possible harboured-genes with adverse effects that would eventually bring about varietal deteriorations.

Maintenance breeding is, therefore, absolutely essential for sustainable production of the certified seed as far as the variety is in commercial production. Note that the maintenance of released varieties and the production of breeder and pre-basic seeds thereof is

the responsibility of the breeding institutions that have developed the varieties. Without implementation of properly-planned maintenance breeding using appropriate techniques, deterioration of varieties is ominous as affinity of pollinating-insects particularly honeybees to these crops would be impressively high both in breeding nurseries and maintenance blocks. The consequence of lack of an acceptable maintenance breeding scheme is typified by the change noted in the seed-color of the variety *Yellow Dodola* of Ethiopian mustard as discussed above, which was pure yellow at the time of its release in 1982 (Belayneh and Alemayehu, 1986), but became almost half-in-half, a mixture of yellow and brown seeds later. Similar change of seed color from white to a mixture of black and white in sesame variety *Adi*, once again signifying the need for handling often cross-pollinated oilseeds differently from the way breeders handle strictly self-pollinated crops in both breeding nurseries as well as maintenance fields.

The same insect-proof breeding cages (Figure 2) being used by the faba bean breeding program at Holetta Agricultural Research Center is found to be effective in excluding pollinating-insects also in maintenance breeding of released varieties of other often cross-pollinated crops with the same style of outcrossing. Another alternative procedure, not commonly yet exploited, is to locate the seed stock for maintenance breeding in the

middle of breeder, breeder seed in the middle of pre-basic and pre-basic seed in the middle of basic seed fields of the same variety so that any rare cross-pollination that could occur or mechanical mixture that takes place will be between the maintenance stock and early generation seed fields of the same variety (Keneni *et al.*, 2001).

Pollination control in early generation seed multiplication

With often-cross-pollinated crops, strict measures have to be taken to avoid random transfer of foreign pollen from neighboring plants or plots that would result in genetic deterioration during maintenance breeding. Even though insect-proof breeding cages were found to be appropriate for faba bean maintenance breeding in Ethiopia, they may not be the best option for early-generation seed multiplication where larger areas are required to produce adequate amount of source material for certified seed production. Therefore, a number of other alternative methods may need to be sought to exclude insect pollinators in early generation seed multiplication.

Temporal isolation is one of the alternative techniques for multiplying seeds of elite varieties with less or no risk of genetic contamination arising from uncontrolled cross-pollination. With this technique, different varieties of same crop are planted in the same season in such a way that their flowering time will not overlap.

Another possibility is of temporal isolation is production of one variety of a crop with rainfall during the main-season followed by production of another variety of the same crop with irrigation during the off-season as practiced by different research centers in Ethiopia. Spatial isolation is another technique that can be used to circumvent contamination from unintended cross-pollination by growing varieties in distances sufficiently away from sources of potential contaminants. This, however, nowadays has become difficult in Ethiopia as land, especially in research centers, has become very scarce.

Growing a given variety of an often cross-pollinated pulse crop, like for instance faba bean, surrounded by a variety of an often cross-pollinated oilseed crop, like for example Ethiopian mustard or rapeseed, works very well for early-generation seed multiplication. Since both species are irresistible to the honeybee, which is the major pollinator for both, when the pollinators first visit the species on the border, they lose any foreign pollen of another variety of the same crop they foraged from elsewhere (Wicombe, 1982). Knowledge of flower preference of pollinating insects maybe helpful in determining the best crop to encircle the main crop for which seed is produced, thereby sieve out pollinating insects, as they commonly show flower preferences (Reverté *et al.*, 2016).

Conclusions and Recommendations

Often cross-pollinated pulse and oilseed crops are definitely playing important economic and ecological roles in Ethiopia. However, failure to control the outcrossing occurring in these crops has been hindering progress that could potentially be achieved in variety development, maintenance breeding and early generation seed multiplication. This review paper has unequivocally revealed that failure to control cross-pollination in often cross-pollinated pulse and oilseed crops in Ethiopia has tremendous drawbacks not only in developing new varieties but also in their maintenance breeding and multiplication of early generation seeds of the varieties.

The economic loss that has so far been incurred in Ethiopia due to failure to control cross-pollination in developing varieties, maintenance breeding and early generation seed multiplication of often cross-pollinated pulse and oilseed crops is apparently difficult, if at all possible, to estimate. Nevertheless, in a situation where outcrossing may reach up to 50%, unless and otherwise fast technical and managerial actions are taken to shortly reverse the situation, we would continue to indignantly lose the virtues of crop varieties. The failure to stop incursion of undesirable traits via uncontrolled cross-pollination in the process of genetic manipulation of

these crops has no justification as there are options of innovative approaches and techniques that breeders can muster to preclude their adverse consequences.

The following recommendations are proposed in a bid to evoke breeders of often cross-pollinated pulse and oilseed crops to reconsider some of their breeding techniques so that by amending any covert flaws of these techniques, they can impart effective and efficient breeding methods to be employed for developing and maintaining varieties and multiplying early generation seed of these crops in the future:

- Breeders and seed producers of varieties derived from often cross-pollinated crops need not allow uncontrolled inter-crossing among genotypes of different genetic background; instead should strictly follow established methods of preventing inter-crossing such as hand emasculation and pollination; use of physical barriers like bagging and caging and spatial and temporal isolations;
- Breeders of often cross-pollinated pulse and oilseed crops in Ethiopia should be cautious of the genetic consequence of uncontrolled outcrossing that these crops are intrinsically prone to if they are treated like self-pollinated crops. Therefore, in order to fully realize the benefits of new releases of varieties of the crops, a true-to-type seed has to be produced starting right from nucleus seed through to certified seed in quantities sufficient to permit perpetual production of the varieties at commercial scale. For this to be true, nucleus/breeders should essentially be concerned about maintaining the genetic integrity of their varieties all along from breeder and/or pre-basic seed level to basic and/or certified seed levels, albeit their primary concern is to develop superior new varieties;
- Information on the amount of cross-pollination and its impact on traits of interest in genotypes of different often cross-pollinated crops grown under different environments representing spatial and temporal variations is generally scanty and thus the extent of out-crossing occurring in the different species of often cross-pollinated pulse and oilseed crops in Ethiopia has to be established as an initial inquiry to prevent the undesirable consequences of uncontrolled outcrossing; and
- Since some of the facilities suggested under this disquisition, like insect-proof breeding cages to curtail free-flow of pollens from every possible alien source and cold stores for keeping stocks of remnant seeds, require a modest investment, research institutions need to allot judicious amount of financial resource; and enforce technical breeding and

seed multiplication standards that need to be observed for the breeding programs of the crops.

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Simple Sequence Repeat (SSR) Polymorphism among Finger Millet Accessions Evaluated for Blast Disease (*Magnaporthe oryzae*) Reaction

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Abstract

A total of 134 finger millet accessions were evaluated in the field for blast disease (*Magnaporthe oryzae*) reaction and also characterized using a set of 20 simple sequence repeat (SSR) markers. The test accessions were mainly from Ethiopia, Eritrea, Kenya, Zambia and Zimbabwe. Based on the head blast disease severity index, only one accession (Acc. BKFM0031) was resistant, 8 accessions were moderately resistant and the remaining 125 fall in the range of moderately susceptible to highly susceptible. SSR based clustering using weighted neighbor joining method grouped the 134 accessions into three major clusters apart from the main node, and the grouping was independent of agro-ecological proximity or similarity in response to blast disease reaction. Analysis of population structure grouped the 134 finger millet accessions into four subpopulation ($k=4$). The first, second, third and fourth group comprised accessions from the different geographical locations with variable level of reaction to blast disease and with membership proportion of 36.5%, 14%, 13.1% and 36.3%, respectively. The present study revealed that finger millet accessions collected either from the same region of origin or with similar level of reaction to blast disease did not often grouped entirely together within a given major cluster or sub cluster constructed using the 20 SSR molecular markers. Therefore, it can be suggested that molecular markers should be identified specifically for finger millet blast disease (*Magnaporthe oryzae*) resistant gene.

Keywords: Area Under Disease Development Curve (AUDPC), Finger millet (*Eleusine coracana* subsp. *coracana*), polymorphism, population structure

Introduction

Finger millet is one of the most important subsistence crop of Africa and India and the major staple food for millions of peoples inhabiting in the arid and semi-arid regions of East Africa (Ekwamu, 1991). Archaeological records revealed that

the primary center of origin for finger millet is East Africa, particularly Ethiopia (Purseglove 1972). This implied that wider genetic variability is expected among Ethiopian accession for blast disease resistance and other agro-morphological traits and thus represents an important resource for the study of finger millet genetics and genome evolution. In Ethiopia, the

area covered by the crop was slightly increasing. For instance, it was produced on 454,662.33 ha in 2013/14 (CSA 2014) but increased to 456057 ha in 2017/18 (CSA, 2018). The productivity increased from 1.87 t ha⁻¹ in 2013/14 to 2.26 t ha⁻¹ in 2017/18.

Genetic diversity is expressed as the genetic differences between species, sub species, varieties, population or individuals. Species with greater genetic diversity are more likely to be able to evolve in response to a changing environment and sporadic pests than those with low genetic diversity (Hunter, 1996). Assessment of genetic variation of a crop species is essential for effective utilization of germplasm in plant breeding programs (Assefa *et al.*, 2001).

Phenotypic characterization is an important first step in the assessment of genetic diversity, but has certain limitations due to morphological plasticity and parallel evolution. These characters are strongly influenced by environmental conditions and show little variation at the intra-specific level (Sangwan *et al.*, 2001). The rapid developments in biotechnology allow easy analysis of a large numbers of loci distributed throughout the genome of plants. Microsatellites or simple sequence repeats (SSRs) are the widely used molecular markers among the others. Due to their high polymorphism (Kubik *et al.* 2009), SSR markers can be used to determine genetic distance between genotypes

and identify parental lines for new variety development with maximum hybrid vigor. SSR markers, therefore, remain markers of choice for practical breeding applications, particularly in developing countries (Sharma *et al.* 2010).

Blast disease caused by *Magnaporthe oryzae* (anamorph *Pyricularia grisea*), has been identified as the highest priority constraint to finger millet production in East Africa (Lenne *et al.* 2007; Takan *et al.* 2011). It can affect all aerial parts of the plant in wet and humid environments. However, several authors reported that the most severe yield losses occurred from panicle infection (Lule *et al.*, 2014; Ekwamu, 1991; Kenneth and LeRoy, 1977). Genetic variation for disease resistance in natural populations is usually estimated by quantitative variation in visual symptoms (Burdon, 1995). Exploiting the genetic variability existing in the working germplasms is vital in the development of resistant or tolerant cultivars. Therefore, the present study was aimed to assess genetic diversity using SSR markers among finger millet accessions evaluated for blast disease for further utilization in cultivar development.

Materials and Methods

Experimental accessions and testing locations

Ninety-nine finger millet accessions collected from major finger millet

producing regional states of Ethiopia (Tigray, Amhara, Oromia, Benishangul Gumuz and Southern Nations-Nationalities and People's Regional States) supplemented by 27 accessions introduced from Eritrea, Kenya, Zambia and Zimbabwe (Table 3) through Ethiopian Biodiversity Institute (EBI) were grown in the 2011 main cropping season under field conditions at Bako Agricultural Research Center (BARC) (1650 m.a.s.l, 7°17'12.5"N; 38°25'47.5"E),

259 km west of Addis Ababa. Seven improved varieties and one advanced line were included in the study. The design was simple lattice with plot size of 9 m² (single row of 1.5m long and 60 cm between row spacing). Spacing between plants within rows were adjusted to 10cm. Fertilizer rates of 105 kg ha⁻¹ DAP at planting and 65 kg ha⁻¹ Urea in split form were used. Table 1 reveals the five years weather condition of the test environment.

Table 1. Weather data of Bako Agricultural Research Center (2009-2013)

Year	Annual RF (mm)	Min temp (°C)	Max temp (°C)	RH (%)
2009	1050.4	12.5	27.5	48.3
2010	1270.5	14.3	28.6	55.2
2011	1425.3	15.8	26.4	60.5
2012	920.6	16.4	29.6	59.4
2013	1435.3	14.2	30.4	49.7

[Source: Bako Agricultural Research Center Agro-meteorology Research Team]

Data Collection and Analysis for head blast disease reaction

The trial was conducted on a sick plot where finger millet was grown as precursor crop in the previous season. Spreader row and artificial inoculation were also employed by developing the inoculums on Potato Dexterous Agar (PDA) following protocols described by Tredway *et al.* (2003). Inoculation/infection was done during humid and cloudy conditions (Han *et al.*, 2003) at early heading and peak vegetative stage. Ten plants per row were randomly selected and tagged prior to heading for plant-based disease assessment. Head blast scores were calculated as the number of

diseased plants divided by the total number of plants sampled in a plot and multiplied by 100 (IBPGR, 1985). Head blast disease assessment was made every two weeks commencing from 102 days after planting (soon after disease appearance on the heads) to physiological maturity. Accordingly, head blast severity was recorded four times. Severity score from ten selected plants were converted into a severity index or disease index as:

$$DI_j = \frac{[\sum_{i=1}^n S_i] \times 100}{n \times 9}$$

where DI_j = disease index at jth round score, S_i = the severity recorded from ith sample plant, n = total number of sample plants considered per plot and

9 = maximum value of scoring scale and is constant. The disease indexes obtained during the different assessment periods were used to calculate the area under disease progress curve (AUDPC) of the

recording period. AUDPC used to quantify and summarize the severity of disease over time was estimated as per the equation suggested by Shaner and Finney (1977) as:

$$AUDPC = \sum_{i=1}^{ni-1} \left\{ \frac{(Y_{xi+1} + Y_{xi})}{2} (t_{i+1} - t_i) \right\}$$

where AUDPC = area under disease progress curve, Y_{xi} = disease severity index recorded for x-accession at i^{th} assessment period; t_i = time interval of disease recording for the assessment period. The sum total of disease progress during the different assessment periods were used as the final AUDPC at the end of the epidemiological period. The estimates of AUDPC was normalized by dividing with the total area of the graph (i.e. the number of days from first appearance of the disease till end of the observation period) in order to facilitate a better visual comparison among host genotypes over season of testing (Shtienberg *et al.*, 1990; Fry 1968). In the present study, the number of days from first appearance/recording of the disease to the end of the observation period was 45. The normalized AUDPC was referred to as the Relative Area under Disease Progress Curve (RAUDPC) and this proportion was employed for different analysis packages used in the study. The RAUDPC were further converted to 1-9 scale (IBPGR, 1985), where 9 represent 100% RAUDPC. Head blast progress during the epidemic periods at regional/country

and varietal level was indicated on monographs generated using Microsoft Excel.

Diversity analysis using SSR markers

DNA extraction

DNA was extracted from field-grown young leaf samples according to the modified CTAB protocol of Mace *et al.* (2003), omitting the phenol: chloroform step. Extracted DNA was visualized on a 0.8% (w/v) agarose gel and quantified spectrophotometrically using a Nanodrop® 1000 (Thermo Scientific, USA), followed by dilution to 10 ng/μl in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0).

Polymerase Chain Reaction (PCR)

The PCR amplification was performed in 10 μl in 384 well microtitre plates and each reaction comprised 1 x PCR buffer (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5% (w/v) Triton X-100; 50% (v/v) glycerol), 2 mM $MgCl_2$, 0.16 mM dNTPs, 0.16 μM fluorescent labeled M13-forward primer, 0.04 μM forward

primer, 0.2 μ M reverse primer, 0.2 units of Taq DNA polymerase (SibEnzyme Ltd, Russia) and 30 ng of template DNA. The PCR reactions were performed on a GeneAmp 9700 thermocycler (Applied Biosystems), with initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute, extension at 72°C for 2 minutes and final elongation at 72°C for 20 minutes.

Fragment detection

DNA samples were subjected to genotyping using 20 published SSR markers (Table 2) for finger millet (Dida *et al.* 2007). All forward primers contained an M13-tag (5'-CACGACGTTGTAAAACGAC - 3') on the 5' end that was fluorescently labeled to allow detection of amplification products (Shuelke, 2000). Amplification was confirmed by running 4 μ l of the PCR products on a 2% (w/v) agarose gel stained with GelRed® (Biotium, USA) and visualized under UV light. Amplification products (1.5 μ l – 3.5 μ l of each) were co-loaded in sets of 3 to 4 markers together with the size standard GeneScan™ –500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems), and separated by capillary electrophoresis

using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems). Allele calling was performed with GeneMapper 4.0 (Applied Biosystems).

Data analysis for SSR Marker

Neighbor-joining-based cluster analysis

The weighted neighbor-joining-based clustering was conducted using DARwin v.5 software (Perrier and Jacquemoud, 2006).

Population structure

Structure version 2.3.3 software (Pritchard *et al.* 2000) was used to establish the number of hypothetically distinct populations. Optimum number of subpopulations was inferred by running an admixture ancestry model with correlated allele frequencies starting from $k = 2$ to $k = 8$, with 10 independent runs at each k . For each run, 5000 burn-ins, followed by 50,000 Markov chain Monte Carlo (MCMC) simulations, were performed. Accordingly, a k value of 4 groups (subpopulations) was selected to describe the genetic structure of the 134 finger millet accessions.

Table 2: List of SSR markers used in this study with repeat motifs and primer sequences

Primer	Forward primer sequence	Reverse primer sequence	Repeat motif	Mapped
UGEP05	TGTACACAACACCACACTGAT	TTGTTTGGACGTTGGATGTG	(TC) ₁₂ AC(TC) ₄	9B
UGEP20	GGGGAAGGCAATGATATGTG	TTGGGGAGTGCCACAATAC	(GA) ₂₀	ND
UGEP27	TTGCTCTGAGGTTGTGTGTTGC	TCAAGCATAGTGCCCTCCTC	(GA) ₁₉	ND
UGEP24	GCCTTTTGATTGTTCAACTCT	CGTGATCCTCTCCTCTCTG	(GA) ₂₆	3B
UGEP12	ATCCCCACCTACGAGATGC	TCAAAGTGATGCGTCAGGTC	(CT) ₂₂	8B
UGEP84	GGAACTTCCGTCAGTCCTT	TGGGGAAGGTGTTGAATC	(CT) ₂₄	ND
UGEP96	TAATGGGCCTAATGGCAATG	CAAATCCGAGCCAAGATTC	(CT) ₁₀	ND
UGEP98	GTCTTCCATTTGCAGCAACC	ACGCGTACTGACGTGCTTG	(GCC) ₈	ND
UGEP67	CTCCTGATGCAAGCAAGGAC	AGGTGCCGTAGTTTGCTC	(TC) ₂₂ TT(GT) ₅	ND
UGEP79	CCACTTTGCCGCTTGATTAG	TGACATGAGAAGTGCCCTGC	(CT) ₁₂	ND
UGEP33	TAGCCGTTTGCTTGTTGTTTTG	AAGGCCCTAGAACGTCAAGC	(TC) ₁₈	ND
UGEP46	CAAGTCAAAACATTGAGATGG	CCACTCCATTGTAGCGAAAC	(GA) ₁₄	ND
UGEP53	TGCCACAACGTCAACAAAAG	CCTCGATGGCCATTATCAAG	(AG) ₂₆	2A
UGEP57	CCATGGGTTTCATCAACACC	ACATGAGCTCGGTATTGC	(AG) ₁₆	ND
UGEP64	GTACAGTCGATTGGAGTGTG	TCTCACGTGCAATTTAGTCAT	(CT) ₂₃	ND
UGEP66	CAGATCTGGGTAGGGCTGTC	GATGGTGGTTTCATGCCAAC	(AG) ₂₉	ND
UGEP95	AGGGGACGCTTGAGTTTG	GCCTCTACCTGTCTCCGTTG	(TC) ₁₄	ND
UGEP73	GGTCAAAGAGCTGGCTATCG	ACCAGAACCGAATCATGAGG	(CT) ₄ CC(CT) ₁₀	ND
UGEP106	AATTCCATTCTCTCGCATCG	TGCTGTGCTCCTCTGTTGAC	(AC) ₁₂	9B
UGEP110	AAATTCGCATCCTTGCTGAC	TGACAAGAGCACACCGACTC	(CT) ₁₂	7AB

Key: ND=not done, B=B genome, A= A genome, AB=both A & B genome of *E. coracana* subsp *coracana* (Dida *et al.* 2007)

Results and Discussion

Epidemiological patterns of blast disease infection

Blast infectivity showed wider variation among accessions of various regions of collection and the improved varieties at early development stage, but the range gets narrow at later recording periods (Fig 1). The infection rate progressed consistently throughout the growing period for the majority of accessions collected from the different corners of the countries (Fig 1) except that accessions from Oromia, Benishangul Gumuz, Zambia, the improved variety Bereda and the advanced line PW-001-022 showed little infectivity at early heading stage (Fig 1). The minimum infections

observed might be attributed to the late maturing characters of some of the accessions in the group.

All aerial parts of the plant were affected by blast disease. However, head/panicle infection causes the most severe yield losses (Lule *et al.*, 2014; Ekwamu, 1991; Kenneth and LeRoy, 1977). Accordingly, based on head blast disease, the total accessions were grouped into 5 classes, where Acc.BKFM0031 collected from Oromia (1368 m.a.s.l.) are the only resistant accession with 20.33% RAUDPC (AUDPC = 915%-day, severity score 1.95), but three accessions collected from Northern Ethiopia showed RAUDPC of 100% (AUDPC = 4500%-days, severity score 9) (Table 3). Similarly, 14 accessions were moderately resistant

with blast infection ranging from 4-5 scale and RAUDPC ranging from 35-55%, 21 accessions were moderately susceptible with blast severity infection 5-6 scale, 21 accessions were susceptible with severity 6-7 and the remaining 77 accessions were highly susceptible with 7-9 severity scale and RAUDPC >78 percent (Table 3).

Similarly, Gashaw *et al.* (2014) evaluated two improved finger millet varieties (Tadesse and Boneya) and one local accession for blast reaction and none of them were resistant. Thakur *et al.* (2009) evaluated 432 different pearl millet lines for blast resistance and found 25 lines consistently resistant to the disease.

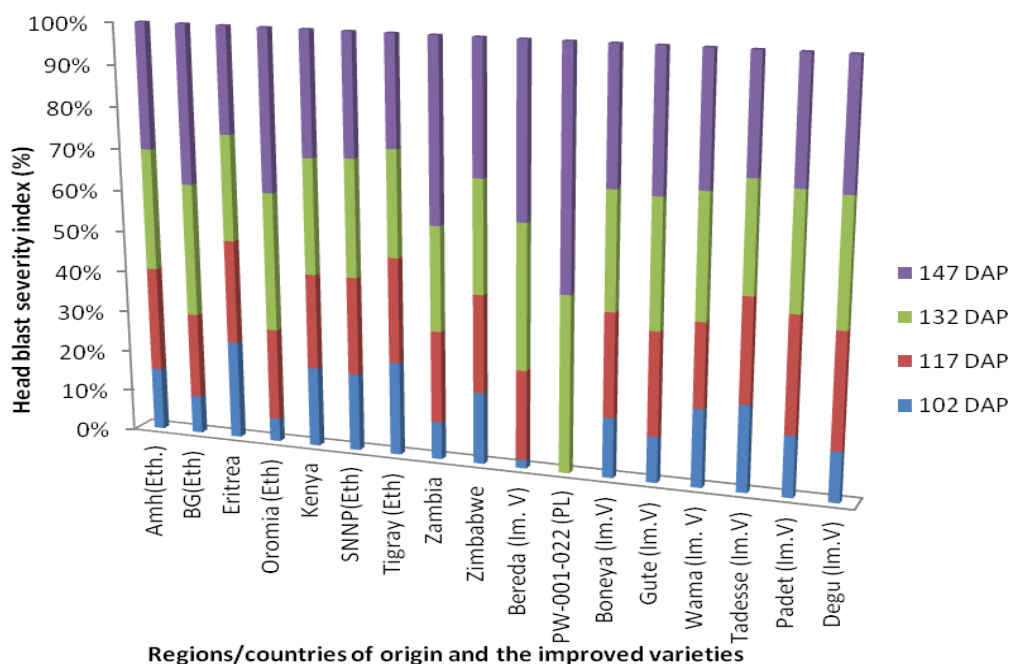


Fig 1. Patterns of head blast severity during the different assessment periods based on regional mean values. Key: DAP= Days After Planting

In the present study, it was observed that among 33 accessions (25% of the total accessions evaluated) less infected by head blast, 73% gave optimum grain yields above the average of the base population (11.75 g plant⁻¹) and 27% gave grain yield below the average (Table 3). On the contrary, among the 33 accessions highly affected by head blast, 79% of

them gave grain yields below the average (Table 3). This indicated that grain yield reduction in most of the accessions was due to the severe blast infection. Similarly, Muyonga *et al.* (2000) reported the highest grain yield was recorded from the most resistant finger millet variety and least grain yield was recorded from the most susceptible variety.

Finger millet accessions collected from the same region showed different levels of disease reaction, implying that diversified finger millet accessions are produced within country or region. Accession BKFM0031 was the most resistant of all, but gave low grain yield per plant. This implied the possibility to use such genotypes as a parental line in crossing programs. Similarly, Anon (1959) reported that a poor yielding variety MO 359 from Mozambique; remained immune and hence was a possible parent for breeding of disease resistant varieties.

About 72.8% of the 33 highly infected accessions were from northern Ethiopia (16 from Tigray and 8 from Amhara) (Table 3). However, among 33 better tolerate accessions, 66.7% was from Western Ethiopia (19 from Western Oromia and three from Benishangul Gumuz). Generally, lower disease severities were recorded for some Western Ethiopian collections and Zambian accessions during the whole assessment period (Table 3). This could be either due to the genetic potential or due to their better adaptive reaction to the test environment and the pathogen or the effect of phenotypic plasticity. On the contrary, accessions from Northern Ethiopia, particularly Tigray and introduced accessions from Eritrea were highly infected by the disease (Table 3).

SSR data based neighbor joining

Weighted neighbor-joining based clustering grouped the 134 accessions into 3 major clusters from the main node (Fig. 2) comprising 82, 37 and 15 accessions in the first, second and third cluster, respectively. Collections from all regional states and agro-ecologies of Ethiopia as well as the introduced accessions were distributed across the first two clusters to varying degrees. Majority of the accessions collected from Amhara and Tigray (about 81% each) were aligned in the first cluster and most of them were susceptible to highly susceptible to blast disease (Fig. 2). The third cluster comprised 15 accessions from Oromia, Amhara, Zimbabwe and one released variety padet (RV-88); where 5 were highly susceptible, 5 susceptible, 3 moderately susceptible and 2 moderately resistant.

Generally, neighboring geographical regions or countries, ecological proximity and also similarity in the level of blast infection rate were not manifested in grouping of the 134 accessions based on 20 SSR markers (Fig 2). Similarly, Babu *et al.* (2007) reported that 32 finger millet accessions fingerprinted using 50 Random Amplified Polymorphic DNA (RAPD) markers, were grouped into two major clusters and the grouping was independent of ecological similarity. Bezawuletaw (2011) also reported that a total of 64 finger millet accessions grouped into nine clusters

with no clear-cut separation among accessions related to their origin.

Very close genetic similarities were observed between some of the accessions collected from countries/regions that were geographically far apart. For example, Acc. 100080 of SNNP shared strong similarities with Acc. AAUFM-41 from Tigray; both were susceptible to blast. Few accessions showed eco-geographical proximity on neighbor

joining clustering. For instance, Acc. BKFM0012 from Oromia showed strong similarity with Acc. BKFM0003 from Benishangul Gumuz region and both of them were among moderately susceptible accessions. Cross-boundary farmer-to-farmer seed exchange, migration and gene flow could be the possible reasons for such relatedness. Similar findings were reported for other crops, such as chickpea (Keneni *et al.*, 2011) and sorghum (Ayana and Bekele, 1998).

Table 3. List of 134 finger millet accessions evaluated for blast disease reaction

PS code	Accession	Country /Region	Alt	HC code	HBAUDPC	RHBAUDPC	HB 1-9 scale	Gy (g/plant)
6	BKFM 0031	Eth/Oromia	1368	7	975.00	21.67	1.95	6.78
129	214987	Zambia	1310	219	1597.50	35.50	3.20	13.90
26	PW-001-022	Advanced		36	1830.00	40.67	3.66	18.55
120	203356	Zimbabwe	1420	205	1897.50	42.17	3.80	15.31
53	BKFM0020	Eth/Oromia	1644	76	1901.25	42.25	3.80	16.11
68	214995	Zambia	1130	102	1912.50	42.50	3.83	14.74
21	214997	Zambia	1100	29	1935.00	43.00	3.87	15.85
52	216035	Eth/Oromia	1900	70	1987.50	44.17	3.98	18.02
16	BKFM0018	Eth/Oromia	1667	22	2141.25	47.58	4.28	16.20
57	BKFM0063	Eth/Oromia	1923	85	2227.50	49.50	4.46	23.05
14	216051	Eth/Oromia	1910	19	2250.00	50.00	4.50	13.32
63	BKFM0023	Eth/Oromia	1891	92	2340.00	52.00	4.68	13.77
10	BKFM0007	Eth/B/Gumuz	1456	11	2475.00	55.00	4.95	11.82
42	BKFM0009	Eth/B/Gumuz	1498	58	2475.00	55.00	4.95	18.11
7	BKFM0038	Eth/Oromia	2144	8	2493.75	55.42	4.99	9.06
2	215984	Eth/Amhara	1870	2	2516.25	55.92	5.03	11.20
28	214989	Zambia	1210	38	2568.75	57.08	5.14	21.40
35	216045	Eth/Oromia	1880	47	2640.00	58.67	5.28	10.01
1	BKFM0014	Eth/Oromia	1409	1	2662.50	59.17	5.33	10.93
60	243640	Eth/Amhara	1890	89	2662.50	59.17	5.33	6.68
32	216049	Eth/Oromia	1600	42	2681.25	59.58	5.36	12.47
3	208725	Eth/Oromia	1920	3	2737.50	60.83	5.48	5.04
128	216046	Eth/Oromia	1680	218	2790.00	62.00	5.58	7.59
130	234826	Eth/Oromia		220	2808.75	62.42	5.62	9.28
15	216050	Eth/Oromia	1700	21	2812.50	62.50	5.63	14.80
19	Bereda	Released		27	2842.50	63.17	5.69	11.83
13	BKFM0046	Eth/Oromia	1367	17	2850.00	63.33	5.70	16.26
43	BKFM0003	Eth/B/Gumuz	1455	59	2850.00	63.33	5.70	17.88
46	BKFM0015	Eth/Oromia	1449	63	2925.00	65.00	5.85	18.97
61	BKFM0044	Eth/Oromia	1601	90	2925.00	65.00	5.85	14.52
95	203362	Zimbabwe	1420	157	2925.00	65.00	5.85	16.11
20	208447	Eth/Amhara	1710	28	2943.75	65.42	5.89	9.75
51	BKFM0045	Eth/Oromia	1615	69	2962.50	65.83	5.93	13.46
4	245088	Eth/Oromia	2060	4	2966.25	65.92	5.93	2.94
38	BKFM 0030	Eth/Oromia	1379	52	2981.25	66.25	5.96	16.80
77	203359	Zimbabwe	1420	124	2988.75	66.42	5.98	13.58
133	235834	Eth/Amhara	1920	224	3000.00	66.67	6.00	16.84
36	Gute	Released		48	3033.75	67.42	6.07	7.72
86	214994	Zambia	1160	138	3048.75	67.75	6.10	16.08
118	203361	Zimbabwe	1420	202	3093.75	68.75	6.19	11.70
25	216048	Eth/Oromia	1640	34	3150.00	70.00	6.30	14.03
62	BKFM0012	Eth/Oromia	1420	91	3150.00	70.00	6.30	25.95
45	Wama	Released		62	3168.75	70.42	6.34	15.79
94	100084	Eth/SNNP	1820	152	3187.50	70.83	6.38	14.48
23	100045	Eth/Amhara	1650	31	3225.00	71.67	6.45	6.94
58	BKFM0019	Eth/Oromia	1596	87	3225.00	71.67	6.45	23.74
126	245084	Eth/Oromia	1915	215	3240.00	72.00	6.48	19.56
82	243636	Eth/Amhara	2100	131	3262.50	72.50	6.53	13.05
134	215960	Eth/Amhara	1980	225	3262.50	72.50	6.53	17.75
55	BKFM0059	Eth/Oromia	1730	83	3300.00	73.33	6.60	18.40
41	203547	Kenya	1510	57	3337.50	74.17	6.68	11.63
88	236445	Eth/Oromia	1870	141	3337.50	74.17	6.68	12.70

Continued from Table 3

PS code	Accession	Country /Region	Alt	HC code	HBAUDPC	RHBAUDPC	HB 1-9 scale	Gy (g/plant)
100	203363	Zimbabwe	1420	175	3337.50	74.17	6.68	10.72
39	BKFM0043	Eth/Oromia	1601	54	3427.50	76.17	6.86	18.88
91	214993	Zambia	1340	145	3427.50	76.17	6.86	7.35
111	242643	Eth/Amhara	1750	193	3450.00	76.67	6.90	10.83
81	100070	Eth/Amhara	1900	129	3487.50	77.50	6.98	6.90
40	BKFM0054	Eth/Oromia	1511	56	3506.25	77.92	7.01	7.45
105	203545	Kenya	1590	182	3525.00	78.33	7.05	20.48
124	245092	Eth/Oromia	1954	212	3540.00	78.67	7.08	11.83
33	BKFM0052	Eth/Oromia	2200	43	3562.50	79.10	7.12	17.22
123	238342	Eth/Amhara	1750	211	3562.50	79.17	7.13	12.12
127	215856	Eth/Amhara	1720	216	3562.50	79.17	7.13	9.04
18	BKFM0036	Eth/Oromia	2144	26	3581.25	79.58	7.16	7.76
12	208440	Eth/Amhara	1850	14	3600.00	80.00	7.20	9.68
84	100038	Eth/Amhara	2030	135	3600.00	80.00	7.20	7.84
109	215854	Eth/Amhara	1750	191	3600.00	80.00	7.20	7.99
101	207964	Eth/Oromia		176	3618.75	80.42	7.24	10.27
27	Boneya	Released		37	3637.50	80.83	7.28	15.72
113	242121	Eth/Amhara	1820	195	3637.50	80.83	7.28	13.54
110	234827	Eth/Oromia		192	3656.25	81.25	7.31	5.66
11	208446	Eth/Amhara	1920	12	3675.00	81.67	7.35	8.74
121	237970	Eth/Oromia	1620	208	3675.00	81.67	7.35	7.13
122	242106	Eth/Amhara	1955	209	3690.00	82.00	7.38	10.82
106	203354	Zimbabwe	1420	184	3693.75	82.08	7.39	18.48
103	237969	Eth/Oromia	1930	178	3712.50	82.50	7.43	10.88
132	215849	Eth/Amhara	1820	223	3712.50	82.50	7.43	10.23
31	203352	Zimbabwe	1490	41	3750.00	83.33	7.50	15.82
97	242110	Eth/Amhara	1990	160	3757.50	83.50	7.52	11.70
37	203360	Zimbabwe	1420	49	3787.50	84.17	7.58	14.37
79	243635	Eth/Amhara	2070	127	3787.50	84.17	7.58	12.81
90	203546	Kenya	1620	143	3787.50	84.17	7.58	14.13
98	203351	Zimbabwe	1490	167	3802.50	84.50	7.61	12.26
59	Padet	Released		88	3825.00	85.00	7.65	12.71
87	208730	Eth/Oromia	1900	140	3825.00	85.00	7.65	13.37
108	203544	Kenya	1485	188	3840.00	85.33	7.68	18.36
50	Taddesse	Released		68	3862.50	85.83	7.73	7.05
5	203358	Zimbabwe	1420	5	3900.00	86.67	7.80	21.79
74	AAUFM-41	Eth/Tigray		118	3918.75	87.08	7.84	13.16
30	238304	Eth/Tigray	1950	40	3937.50	87.50	7.88	3.57
73	229726	Eth/B/Gumuz	1600	112	3937.50	87.50	7.88	7.43
96	242625	Eth/Tigray	1880	158	3937.50	87.50	7.88	13.58
99	238343	Eth/Amhara	1970	170	3937.50	87.50	7.88	8.76
67	235145	Eth/Tigray	1730	100	3956.25	87.92	7.91	6.47
22	237445	Eth/Tigray	1940	30	3975.00	88.33	7.95	6.16
49	242107	Eth/Amhara	2020	67	3975.00	88.33	7.95	5.76
125	234825	Eth/Oromia		214	3975.00	88.33	7.95	8.17
44	215886	Eth/Amhara	1850	60	4012.50	89.17	8.03	10.88
89	234199	Eth/Tigray	1920	142	4012.50	89.17	8.03	11.18
117	Degu	Released		200	4031.25	89.58	8.06	4.38
34	215867	Eth/Amhara	1990	45	4050.00	90	8.10	8.79
54	237446	Eth/Tigray	1850	81	4050.00	90.00	8.10	5.00
17	229727	Eth/B/Gumuz	1450	25	4087.50	90.83	8.18	6.83

Continued from Table 3

PS code	Accession	Country /Region	Alt	HC code	HBAUDPC	RHBAUDPC	HB 1-9 scale	Gy (g/plant)
47	AAUFM049	Eth/Tigray		64	4087.50	90.83	8.18	11.15
78	AAUFM-29	Eth/Tigray		126	4087.50	90.83	8.18	6.48
56	238305	Eth/Tigray	1990	84	4125.00	91.67	8.25	13.89
104	203350	Zimbabwe	1400	179	4125.00	91.67	8.25	8.04
112	215863	Eth/Amhara	1850	194	4125.00	91.67	8.25	11.73
48	AAUFM-3	Eth/Tigray		66	4162.50	92.5	8.33	4.99
66	203355	Zimbabwe	1420	98	4162.50	92.50	8.33	9.14
76	215865	Eth/Amhara	1880	122	4185.00	93.00	8.37	10.25
92	245085	Eth/Oromia	1987	146	4200.00	93.33	8.40	11.73
9	242623	Eth/Tigray	1590	10	4237.50	94.17	8.48	11.09
80	AAUFM-28	Eth/B/Gumuz		128	4237.50	94.17	8.48	17.85
115	203542	Kenya	1540	198	4260.00	94.67	8.52	14.65
24	242619	Eth/Tigray	1810	33	4275.00	95.00	8.55	8.20
29	230101	Eritrea	1740	39	4312.50	95.83	8.63	10.73
69	AAUFM-10	Eth/Tigray		103	4312.50	95.83	8.63	5.24
70	208439	Eth/Amhara	1950	108	4312.50	95.83	8.63	5.77
83	AAUFM-17	Eth/Tigray		133	4312.50	95.83	8.63	14.75
102	215853	Eth/Amhara	1780	177	4312.50	95.83	8.63	8.43
116	215852	Eth/Amhara	1790	199	4312.50	95.83	8.63	8.41
64	AAUFM-050	Eth/Tigray		96	4350.00	96.67	8.70	10.55
107	100002	Eth/Amhara	2004	187	4350.00	96.67	8.70	4.66
119	203357	Zimbabwe	1420	203	4350.00	96.67	8.70	15.92
65	AAUFM-6	Eth/Tigray		97	4350.00	96.67	8.70	5.92
71	AAUFM-21	Eth/Tigray	1722	109	4387.50	97.50	8.78	11.89
131	242115	Eth/Amhara	1890	222	4387.50	97.50	8.78	13.20
8	238299	Eth/Tigray	1940	9	4425.00	98.33	8.85	9.05
72	230105	Eritrea	1600	110	4462.50	99.17	8.93	4.35
85	230104	Eritrea	1800	137	4462.50	99.17	8.93	6.30
75	AAUFM-44	Eth/Tigray		121	4500.00	100.00	9.00	5.58
93	228202	Eth/Amhara		149	4500.00	100.00	9.00	6.46

Key: Alt= altitude, HC SN=serial number labeled for hierarchical clustering, PS=accessions serial number as labeled for population structure analysis, HBRAUDP= head blast relative area under disease progress curve, GYPPL =grain yield per plant

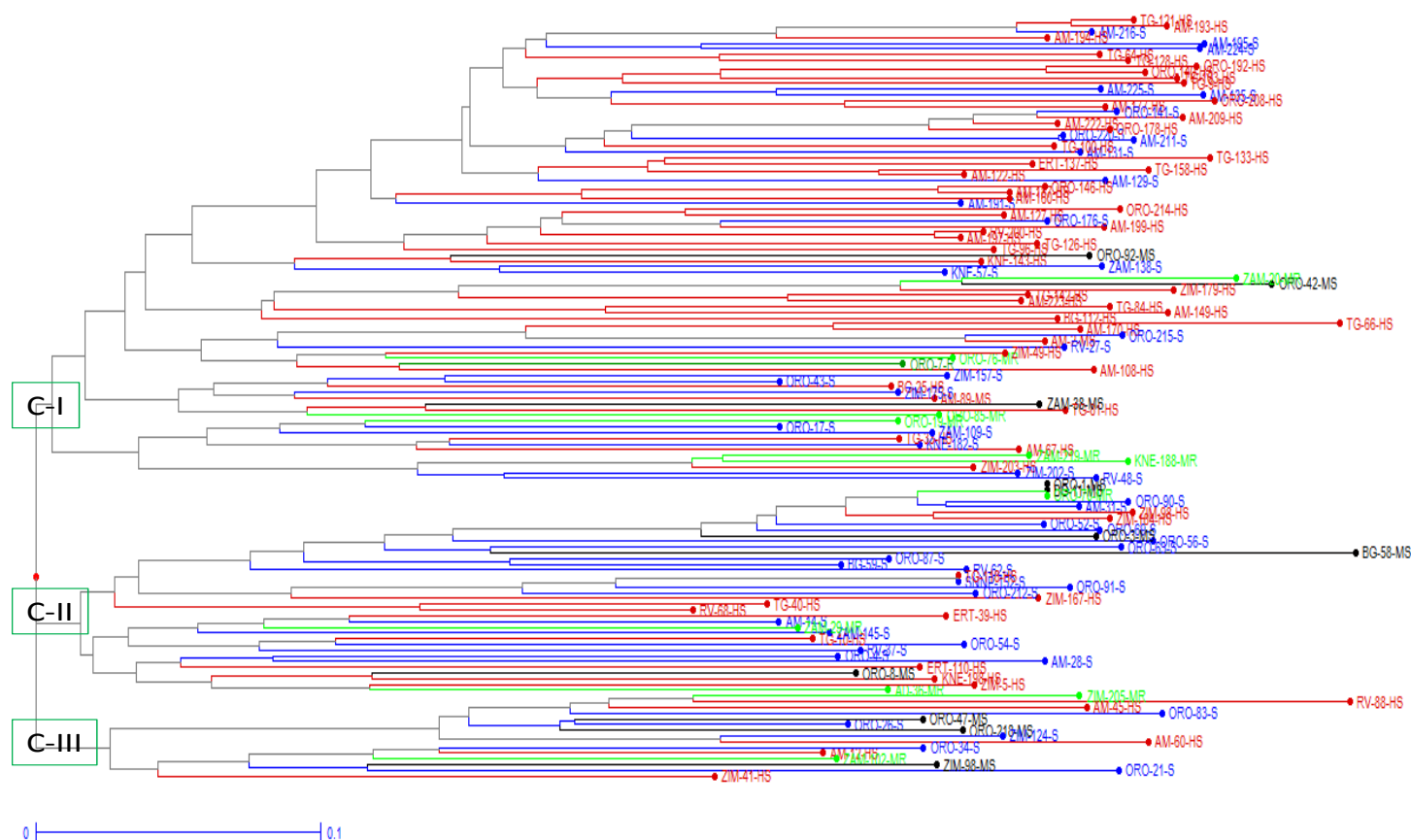


Figure 2. Weighted neighbor joining based clustering of 134 finger millet accessions for 20 polymorphic SSR markers evaluated for blast disease. Key: AM = Amhara, ORO = Oromia, TG = Tigray, BG = Benishangul Gumuz, SN = Southern Nation Nationalities and peoples region, KNE = Kenya, ZAM = Zambia, ERT = Eritrea, ZIM = Zimbabwe, HS=highly susceptible, S=Susceptible, MS=moderately susceptible, MR=moderately resistant, R=Resistant. The number following name of each region on the graph indicates finger millet accessions serial number sequentially as listed in Table 3 coded as HC and evaluated for SSR analysis.

Population structure analysis

Analysis of population structure grouped the 134 finger millet accessions into four subpopulation (groups) (Fig 3). Vanniarajan *et al.* (2012) categorized 31 rice landraces along with 9 indica and japonica test cultivars into three major subpopulations. Kannan *et al.* (2014) reported four subpopulations ($k=4$) for 250 full Sibs progenies of pearl millet. In agreement with neighbor-joining trees, analysis of population structure revealed that either accessions collected from the same region of origin or having similar level of infection to the blast disease did not often group entirely together within a given major group (Fig 3). For instance, among the 34 finger millet accessions having group-I as their largest component, collections from all regions/countries with different levels of infection to the blast disease were aligned together in a varying degree.

Eco-geographical proximity was also partly manifested; for example, 35%

of the accessions having group-1 as their largest component were collected from Tigray; eight of the 12 finger millet accessions having group-3 as their largest component were from Oromia regional state and also most of them were among the moderately tolerant to blast disease. The first group revealed the membership proportion of 36.6%, with an average distance (expected heterozygosity) of 0.5426 between individuals in same cluster (Table 4). The second, the third and the fourth groups also comprised accessions from different geographical locations and the improved varieties with different blast infection rate and the membership proportion of 14%, 13.1% and 36.3%, respectively. The fixation index (F_{ST}) values of the subpopulations ranged from 0.090 (group-4) to 0.365 (group-3) (Table 4). The pair-wise allele frequency divergence values were highest (0.254) between the second subpopulation and the third subpopulation but lowest (0.092) between group-2 and group-4.

Table 4. Membership proportion, expected heterozygosity and F_{ST} of the four subpopulations

Sub-population or group	membership proportion	expected heterozygosity between individual in the same cluster	mean value of F_{ST}
1	36.6%	0.5426	0.1921
2	14%	0.5988	0.2108
3	13.1%	0.4185	0.3654
4	36.3%	0.6288	0.0900

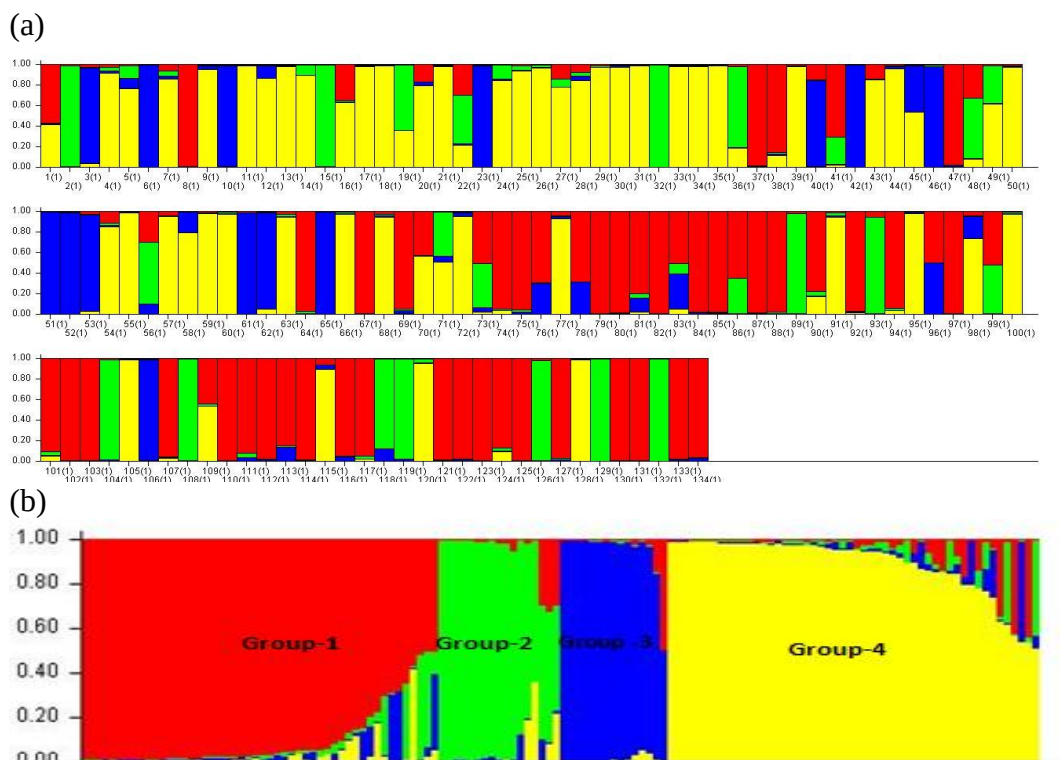


Figure 3: Estimated population structure of 134 finger millet genotypes plotted in a single line with population ID (a) and sorted by ancestor coefficient Q (b) for $k = 4$. The y-axis is the group (subpopulation) membership, and the x-axis is the individual finger millet genotypes. The number under each line indicates finger millet accessions serial number sequentially listed in Table 3

Conclusion

Blast disease, caused by *Magnaporthe oryzae* (anamorph. *Pyricularia oryzae*) is the major pathogen critically affecting the production and productivity of finger millet. Only one accession was resistant and more than 90% of the tested accessions fall in the range of susceptible to highly susceptible. Weighted neighbor-joining based clustering grouped the 134 accessions into 3 major clusters from the main node such that finger millet accessions collected either from the same region of origin or with similar level of blast infection

(resistance level) did not often grouped entirely together within a given major cluster or sub cluster. But very few accessions showed close genetic similarities while they were collected from countries/regions that were geographically far apart and few other accessions manifested eco-geographical proximity in clustering. Analysis of population structure distinguished the 134 finger millet accessions in to four subpopulation ($k = 4$). Similar to the result of neighbor joining trees, finger millet accessions collected from the same region of origin or with similar level of disease resistance did not often grouped entirely together within a given major

groups. This recalls for further development and analysis using molecular markers specifically identified for finger millet blast disease (*Magnaporthe oryzae*) resistant gene.

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Assessment of the Symbiotic Effectiveness and Population Size of Soybean Rhizobia in Ethiopian Soils to Assist Appraisal of Crop Inoculation Requirements

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Abstract

Population density and nitrogen fixation effectiveness of indigenous soil rhizobia are important factors in predicting a response to legume inoculation. Fifty-five soils from major soybean-growing areas of Ethiopia were assessed for abundance and symbiotic effectiveness of soybean-nodulating rhizobia, using the most probable number and whole soil inoculation techniques. Estimated population densities ranged from non-detectable to $>1.5 \times 10^4$ cells/g soil, and 49% of the soils had rhizobial populations of <300 cells/g soil. Shoot dry weight of plants inoculated with soil suspensions ranged between 45 to 142 mg/plant, compared with 60 mg/plant when un-inoculated and 136 mg/plant when inoculated with reference strain (*Bradyrhizobium japonicum* strain CB1809). Comparison of shoot dry weights showed that in soils with populations of >300 cells/g soil, 13% contained ineffective rhizobial populations. Soils from southwestern Ethiopia had larger and more effective rhizobial populations; hence, widespread crop inoculation responses are unlikely. Responses to inoculation would be very likely in the other areas tested, as the soils mostly contained low and/or ineffective rhizobia populations. The study identified regions where extension efforts related to inoculation of soybean would provide the most benefit. Soil properties were not strongly correlated with population density of rhizobia while leaf greenness was a useful predictor of nodulation and symbiotic nitrogen fixation.

Keywords: most probable number, nitrogen fixation, resident rhizobia, soybean, symbiotic effectiveness, whole soil inoculation.

Introduction

The population density and effectiveness of rhizobia resident in a soil are reliable predictors of the need to inoculate legumes (Brockwell *et al.*, 1988, Thies *et al.*, 1991, Furseth *et al.*, 2010). Determining the effectiveness of resident rhizobial populations can be constrained by the perceived need to isolate individual strains of rhizobia assess their effectiveness (Brockwell *et al.*, 1988) or the resources required to conduct agronomic trials (Singleton *et al.*, 1992). Assessment of the symbiotic effectiveness of a population of soil rhizobia using the whole soil inoculation (WSI) technique (Brockwell *et al.*, 1988) can provide a rapid assessment of the entire soil population, so is more likely to represent the response from a genetically or phenotypically diverse population. The WSI, method, coupled with the most probable number (MPN) technique, can provide an assessment of the need for inoculation (Denton *et al.*, 2000), as these combined techniques estimate both density and symbiotic effectiveness of the soil population of rhizobia.

Although studies have recently focused on the genetic diversity of soybean rhizobia (Aserse *et al.*, 2012, Jaiswal *et al.*, 2016), information is lacking on the abundance of resident soybean-nodulating rhizobia pop-

ulation in Ethiopian soils. The absence of nodules on soybean roots in some fields has been observed (Hailemariam, personal communication), however assessment have not been made across all the areas where soybeans are grown. Inoculation responses for soybean have been obtained using imported commercial inoculants in field conditions (Aserse *et al.*, 2012, Bekere, 2012, Jefwa *et al.*, 2014), however inoculation has not consistently improved yield, and failures have been observed in some areas like Bako; the reasons for this are not clear but low soil pH is one possibility (Aserse *et al.*, 2012). It was reported that soil acidity significantly decreased the response of soybean to inoculation in Southwestern Ethiopia (Bekere *et al.*, 2013).

In this study, we assessed the abundance and symbiotic effectiveness of rhizobia in soils sampled from the four major soybean-growing areas of Ethiopia, using the WSI and MPN techniques, respectively. Soils were collected from cultivated fields and nearby uncultivated lands and cropping and inoculation history was established by interviewing farmers. It was hypothesised that low populations and/or low effectiveness of soybean-nodulating rhizobia in Ethiopian soils may limit effective N₂ fixation and production of soybean. In addition, acid soils may limit the survival of

rhizobia and lead to low population densities.

Materials and Methods

Site selection and collection of soil samples

Fifty-five soil samples were collected from four major soybean-growing areas of Ethiopia: (Table S1, Fig.1), Southern Ethiopia (SE, $n = 11$), Southwestern Ethiopia (SWE, $n = 21$),

Western Ethiopia, i.e. areas around Bako to Gute (WE, $n = 16$) and Assosa Zone (AZ, $n=7$). Farmers at most of the soil sample collection sites provided at least two years of cropping and inoculation history of the fields. Soil samples (0-20 cm depth) were taken from four points in each field and were mixed to make one composite sample and placed at room temperature until the experiment was conducted.

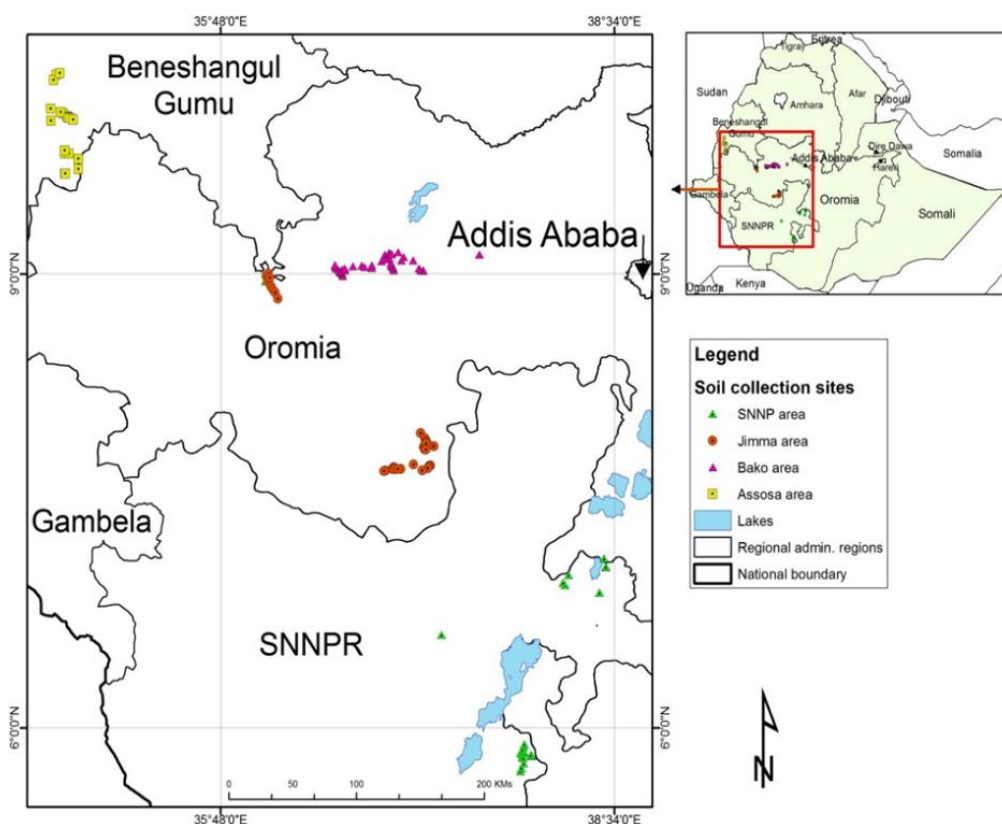


Figure 1. Soil sample collection sites of major soybean growing areas of Ethiopia. Southern Ethiopia represents the Southern Nations and Nationalities People (SNNP) Regional State, south western (represented for short as Jimma and it includes Chewaka area) and western Ethiopia (Bako area

and includes fields up to Gute) represent the Oromia Regional State, Assosa Zone of Benishangul Gumuz Regional State.

Soil analysis

The soil samples collected from the field were air-dried, ground, and passed through a 2mm sieve. All the analysis followed the methods of Rayment and Higginson (1992), specifically; total nitrogen was analysed using a Leco analyser (Method 6B1), pH H₂O (Method 4A1), pH CaCl₂ (Method AB1), electrical conductivity (Method 3A1), nitrate nitrogen and ammonium nitrogen (Method 7C2b), Colwell phosphorus (Method 9B), Bray II P (Method 9E2), organic carbon (Wakeley and Black, Method 6A1), phosphorus buffering index (PBI, Method 912c), texture using mid-infrared (Method 6B4b).

Most probable number determination and whole soil inoculation

The population density of soybean-nodulating rhizobia and nitrogen fixation effectiveness of the soil rhizobial population were determined concurrently in one experiment. To determine MPN of the soils (Woomer, 1994), six levels of serial dilution of each soil (5^{-1} to 5^{-6}) were prepared and each soil dilution was used to inoculate four pots (each with one plant). Symbiotic effectiveness using the whole soil inoculation technique (Brockwell *et al.*, 1988) was determined from the replication of

plants that received the lowest dilution e.g., highest concentration, of the soil suspension (5^{-1}). Soils with <300 cell/g soil are considered as small populations and may not provide an accurate representation of the effectiveness of the rhizobial population, as this number may limit the expression of symbiotic effectiveness (Denton *et al.*, 2000); those categorised as having >300 cells/g soil are considered to be sufficiently large populations.

Pots were prepared using aseptic techniques and the experiment was conducted in a quarantine-approved facility. Approximately 460 g of pre-washed sand and vermiculite (1:1, v/v) was added to each 500-ml pot, moistened with McKnight's nutrient solution (Gibson, 1980) to 60% of the pore space, and for 20 min at 121°C. Soybean (*Glycine max* cv. Soy791) seeds were surface using 95% ethanol for 10s and 3% sodium hypochlorite for 3 min (Somasegaran and Hoben, 1985) followed by rinsing with six changes of sterile water. Seeds were aseptically transferred to pots (4 seeds/pot) and thinned to one plant per pot 7 d after germination.

Soil suspension and inoculation were carried out aseptically. Soil samples (100 g) were suspended in 400 ml of sterilised saline solution (0.89% NaCl) and mixed on a shaker at 150 rpm for

25 min. Each of the soil suspensions was diluted through a six-step fivefold series (5^{-1} to 5^{-6}), transferring 5 ml of suspension to 20 ml of sterile saline solution at each step (Woomer, 1994). For each soil, four pots for each dilution were inoculated with 1 ml of suspension, a total of 1320 pots. A reference strain (CB1809, obtained from the Australian Inoculants Research Group, Gosford, NSW) was grown in yeast extract mannitol broth (Somasegaran and Hoben, 1985) for 7 d and used to inoculate four replicate plants. Uninoculated seedlings with or without nitrogen served as positive and negative controls, respectively. After inoculation, the pots were randomised and the sand surface was covered with sterile 3 mm alkathene beads to reduce water loss and contamination. The plants were grown in a controlled environment with a 12 h photoperiod with day/night temperatures of 25°C/15°C, respectively.

Harvest

The SPAD (chlorophyll concentration) measure was taken from the middle leaflet of the first trifoliate leaf of the plants at 28 d after germination using Chlorophyll meter (SPAD-502 Plus, Konica Minolta Optics, Inc, Japan). Plants were harvested after 6 weeks. The roots were assessed for the presence or absence of nodules, to determine the MPN and its correlation with other growth parameters. The four plants with the first dilution (5^{-1})

were assessed for symbiotic nitrogen fixation effectiveness, relative to the un-inoculated control and the reference strain. During harvest, the nodules were counted, and shoots and nodules were oven dried at 70°C for 48 h before weighing.

Data analysis

The MPN estimate was determined following the procedure previously outlined (Woomer, 1994). The lower and upper confidence interval limits ($p = 0.05$) for each estimate were calculated by dividing and multiplying the respective estimate by 3.40 (Woomer, 1994). The shoot dry weight data of the plants used for whole soil inoculation (the first four plants that received the lowest dilution) were taken and subjected to analysis of variance (ANOVA) using GenStat (VSN International, 2014). The least significant difference (LSD) was used to separate treatment means at 5% level of significance and mean shoot dry weights of the treatments were compared with the un-inoculated plants and/or plants inoculated with CB1809. An isolate was considered effective if the mean value of the dry matter accumulated in the inoculated plants was not significantly different ($P < 0.05$) from that of the CB1809-inoculated host, while being significantly different from the un-inoculated control. An isolate was classified as moderately effective if the performance of the inoculated plant was significantly higher than the un-

inoculated control but significantly lower than that of the CB1809-inoculated host, and ineffective if the dry matter accumulated was not significantly different from that of the un-inoculated control (Abaidoo *et al.*, 2007).

Soil physical and chemical variables were analysed using principal component analysis using XLSTAT (Addinsoft, 2016), to determine whether the soil collection sites form distinct groups based on the soil characteristic data. Their correlation with rhizobial number and effectiveness was also examined.

Results and Discussion

Rhizobial population density with regard to inoculation history and symbiotic effectiveness

This is the first study to quantify the soil population densities of soybean-nodulating rhizobia and their symbiotic effectiveness in Ethiopia covering large areas. The assessment of rhizobial population (numbers) and symbiotic effectiveness from the major soybean-growing areas of Ethiopia is important for the prediction of responses to inoculation (Weaver *et al.*, 1972, Brockwell *et al.*, 1988, Turk *et al.*, 1993) and are therefore important determinants of N₂ fixation, soybean yield and grain protein level.

The density of rhizobial populations in the soils ranged from non-detectable to $> 1.5 \times 10^4$ cells/g soil (antilog $10^{4.18}$). Forty- nine percent of the soils (27 of 55) had a low rhizobial count (< 300 cells/g soil, Table S1). Soils from SWE, WE and AZ had maximum rhizobial populations of $> 1.5 \times 10^4$ cells/g soil, while the maximum density of the rhizobial population for SE was 5.9×10^4 cells/g soil. No rhizobia were detected in two of the 16 soil samples from the Bako area (soils 45 and 51) in WE (Table S1).

The highest proportion of soil samples with large rhizobial populations (> 300 cells/g soil) were typically identified from SWE of the Oromia Regional State, while soil samples from WE had the lowest proportion of soil samples with large rhizobial populations (Table S1). Conversely, the highest proportion of soil samples with small rhizobial populations (< 300 cells/g soil) were found from WE, while the lowest proportion were from SWE. The rhizobial population density was not significantly correlated with either the years since soybean was sown ($R^2 = 0.02$) or history of inoculation ($R^2 = 0.0004$, Table S1).

The symbiotic effectiveness of the soil rhizobial populations was assessed by comparing shoot dry weight of inoculated plants with shoot dry weights of the negative control (without inoculation or nitrogen

addition) and of CB1809-inoculated plants. Inoculation with the soil samples resulted in shoot dry weights from 45 mg (soil 3 from WE) to 142 mg (soil 26 of SWE), whereas the un-inoculated plants and the plants inoculated with CB1809 had means of 62 and 136 mg, respectively. Rhizobial populations in 25% of the soils sampled had less than half of the symbiotic performance of CB1809, while 62% of the surveyed soils had

ineffective rhizobial populations. Soils with <300 cells of rhizobia/g soil (49% of soil samples) did not increase shoot dry weight above that of the un-inoculated plants. The soil samples with >300 cells/g soil (51%) were categorised as having effective (31%), moderately effective (7%) and ineffective (13%) rhizobia (compared to CB1809 and the un-inoculated control; Figure 2).

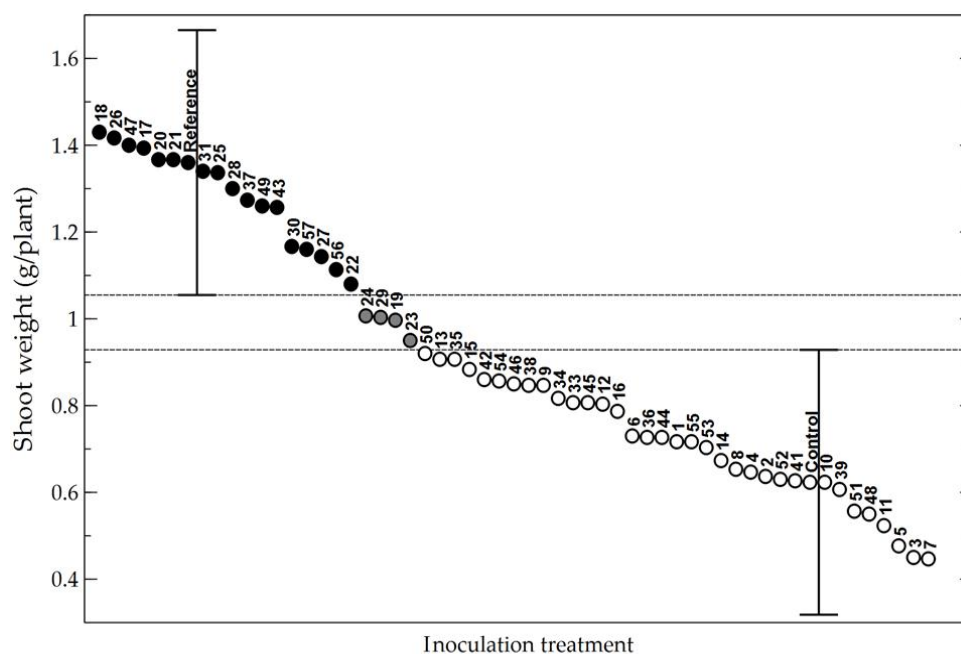


Figure 2. Shoot dry weight for plants inoculated with 55 soil samples suspensions collected from major soybean growing areas of Ethiopia compared to a reference strain (CB1809) and an un-inoculated control. The soil resident populations are divided into three groups: (●) not significantly different from the reference, (○) not significantly different from the control and (◐) significantly different from both the reference and the control. Error bars are the 95% LSD and the dashed lines indicate the boundaries between the three groups.

Soil samples from the different soybean-growing areas differed in the proportion of effective rhizobia populations. SWE soils had the highest frequency of effective (62%) and

moderately effective (24%) rhizobial populations, relative to CB1809, while none of the soils sampled from AZ harboured effective populations. The proportions of soil samples containing

effective rhizobial populations for SE and WE were 2% and 19% respectively (Figure 3).

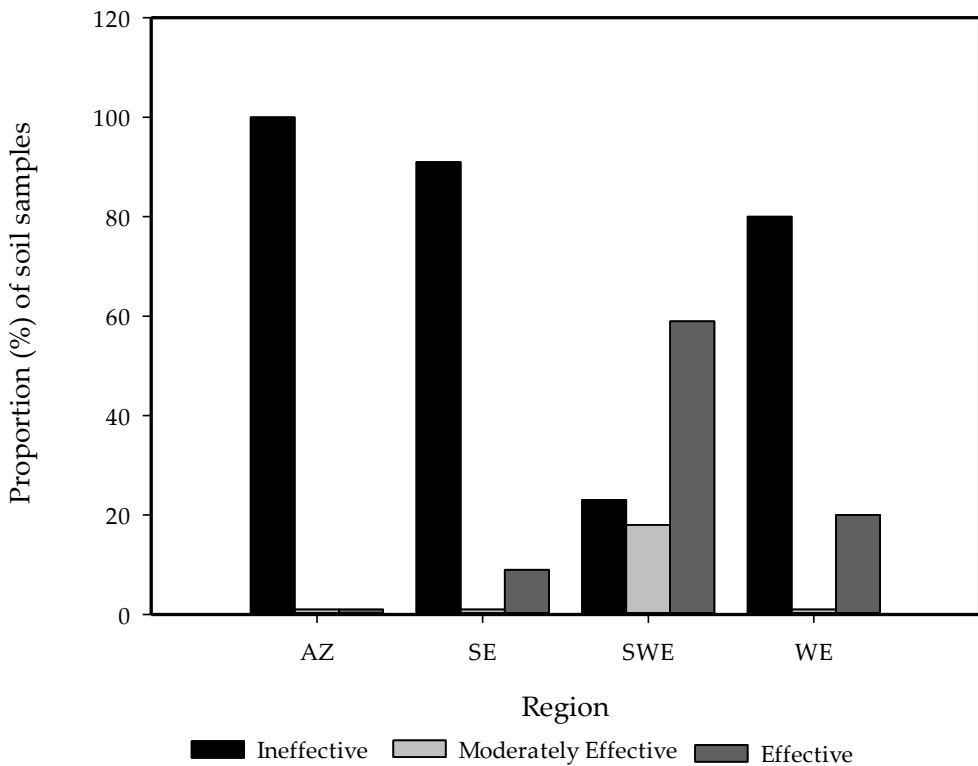


Figure 3. Effectiveness of soil rhizobial populations from different areas of Ethiopia where the rhizobia population was >300 cells/g soil as determined by the most probable number technique. SE, Southern Ethiopia; SWE, Southwestern Ethiopia; WE, Western Ethiopia; and AZ= Assossa Zone in Benishangul Gumuz Regional State.

In this study, 96% of the soil samples assessed contained rhizobia that were able to form nodules on soybean. The soil samples that formed nodules were taken from fields that grew soybean in rotation with other crops, or had grown soybean only once (e.g. soils 9 and 19), or where soybean had not been grown (e.g. soil 36); two fields from natural forests neighboring fields were also tested (e.g. soils 44 and 52). The detection of soybean rhizobia in soils from natural forests and from

soils that had never been cultivated suggests that soybean-nodulating rhizobia are indigenous to some Ethiopian soils, although we are not aware of the native plant species that might nodulate with these rhizobia. Soybean-nodulating indigenous rhizobia, potentially from native legumes, have similarly been found in other African countries, such as Nigeria (Sanginga *et al.*, 1996), Zimbabwe (Musiyiwa *et al.*, 2005) and Zambia (Javaheri, 1996).

Alternatively, soybean rhizobia may have potentially moved from surrounding farmland, potentially via drifting soil particles (Rosselli *et al.*, 2015). The lack of association between rhizobial population density and inoculation history, and the lack of association between rhizobial numbers and the years since soybean was last grown was unexpected. In other studies, stronger relationships between paddock crop rotation history and rhizobial populations were observed (Drew *et al.*, 2012). The weak or lack of the associations might be due to the presence of ineffective indigenous rhizobial populations in the assessed soil samples.

The frequency of detection of soybean-nodulating rhizobia in Ethiopian soils was higher than reported for nine other African countries (Abaidoo *et al.*, 2007), where *Bradyrhizobium* spp. nodulating soybean cv. TGx and *Bradyrhizobium japonicum* nodulating soybean cv. Clark were detected in 72% and 37% of the soils, respectively. In the present work, fivefold dilution series were used to detect rhizobial populations as small as 2 cells / g soil (Somasegaran and Hoben, 1985), and this sensitivity may have contributed to a higher frequency of rhizobial detection.

Despite the relatively high frequency of detection of rhizobia in the present study, 49% of the soil samples had <300 cells/g soil and 42% of soils had

<50 cells/g soil. A high percentage of soil samples with low (300 cell/g soil) numbers of soybean-nodulating rhizobia was found in soil samples from AZ, WE and SE. In these areas with smaller populations of rhizobia, soybean yields will likely be improved through inoculation (Jefwa *et al.*, 2014). Soybean rhizobial inoculation in soils with low rhizobia population densities has previously been demonstrated to improved soybean growth in Nigeria (Ronner *et al.*, 2016), Egypt (Youseif *et al.*, 2014) and Kenya (Thuita *et al.*, 2012, Herrmann *et al.*, 2014). A meta-analysis study on soybean inoculant effectiveness of selected experiments showed that the largest nodulation due to inoculation occurred in soils with no or low background rhizobial population (Thilakarathna and Raizada, 2017). In such cases, inoculation is a cheap and sustainable alternative to increase soybean yield compared with supply of inorganic N or organic inputs, which are expensive for smallholder farmers (Hungria *et al.*, 2013; Ronner *et al.*, 2016).

In contrast to our expectations that assumed soybean is introduced crop to Ethiopia and may lack the associated rhizobial population, larger populations of soybean-nodulating rhizobia were detected in this study. Large populations of rhizobia were detected in 51% of the soil samples in the present study. Ninety five percent of the samples from SWE fall in this

category and 61% of these SWE soil samples contained $>10^4$ cells/g soil. This is high compared with a previous report in which only 18% of soils from Iowa that had never grown soybean contained $>10^4$ soybean-nodulating rhizobia/g soil (Weaver *et al.*, 1972). The maximum rhizobial count reported for soybean in central India was also only 3.3×10^3 cells/g soil (Ansari and Rao, 2014). The presence of indigenous or naturalised rhizobial populations is a significant factor that can affect the response to inoculation (Thies *et al.*, 1991; Brockwell *et al.*, 1995; Slattery and Pearce, 2002; Denton *et al.*, 2007; Denton *et al.*, 2013). In particular, the presence of even 50 cells of rhizobia/g soil was able to suppress the response to inoculation in some soils (Thies *et al.*, 1991) and soil populations have been shown to reduce the success of nodule occupancy by commercial inoculants (Denton *et al.*, 2002). Variable numbers of rhizobia in soils identified in the present study may account for some inconsistent responses to inoculation observed with commercial inoculants of soybean in Ethiopia (Aserse *et al.*, 2012).

Understanding the need to inoculate legumes, and how this varies geographically, provides useful information regarding the likelihood of responses expected from inoculation. Hence, according to our results, inoculation is unlikely to increase yields in many soils in SWE unless a

significantly more effective and competitive strain is used, or isolated areas with low population numbers are identified ahead of inoculation. In contrast, inoculation is much more likely to be much more important in Assossa in particular (as 71% of the samples contained <100 cells/g soil with no effective populations present), and in WE and SE. Farmers in this region should therefore be advised to inoculate their soybean crops and appropriately targeting these regions through extension would be more worthwhile than efforts to improve soybean through inoculation in the SWE region.

With regard to symbiotic nitrogen fixation effectiveness, 62% of the soil samples from SWE contained effective populations of soybean rhizobia. It is likely that the soils from this region can be a good source of inoculants, as demonstrated by the greenhouse effectiveness test in which 62% of the soils from this region had similar effectiveness to the reference strain. Effective indigenous populations have been detected in other African soils, e.g. in Nigeria (Sanginga *et al.*, 2000) and Zimbabwe (Musiyiwa *et al.*, 2005). However, for SE and WE regions of our study, the proportions of effective rhizobial population were lower (2 and 5%, respectively), and not detected at all in soils from AZ. Studies in Sichuan province of China also identified that 89% of strains

isolated were either ineffective or poorly effective (Chen *et al.*, 2015).

Shoot dry weight in the absence of nitrogen supply was equated to symbiotic effectiveness, and was positively correlated with both numbers of rhizobia/g soil, and nodule numbers in the present study, indicating the beneficial impact of indigenous rhizobia in soil on soybean growth responses. Although rhizobia population density was positively correlated to shoot dry weight in the present study ($p < 0.001$), the correlation was not strong ($R^2 = 0.34$), which can be explained by the occurrence of large populations of rhizobia with low symbiotic effectiveness (e.g. soil 3 in WE, soil 4 in AZ, soils 1 and 5 in SWE). The low correlation between nodule number and shoot dry weight ($R^2 = 0.23$) indicates the presence of potentially ineffective soil rhizobia that were able to nodulate soybean but not fix nitrogen effectively. In the present study, the positive correlation between SPAD reading and shoot dry weight ($R^2 = 0.40$) indicates that SPAD readings could assist in the selection of plants that have nodules containing effective rhizobia, without the need to harvest plants or to collect, dry and process dry matter, or at least to prioritize selection within large screening efforts. Effective N_2 fixation is expected to lead to increased leaf chlorophyll, and result in increased photosynthesis (Mabood and Smith,

2005) which in turn should increase plant biomass, as observed in the present study.

Associations among measured variables

Rhizobial density (MPN) was positively correlated with shoot dry weight ($R^2 = 0.33$, $p < 0.001$, Figure 4). Nodule number per plant ranged between 0 (soils 45 and 51, and the un-inoculated control) and 69 (soil 49), and was positively correlated with both greenness (SPAD reading) ($R^2 = 0.50$, $p < 0.001$, Figure 5) and shoot dry weight ($R^2 = 0.23$, $p < 0.001$, data not shown). The SPAD reading was also positively correlated with shoot dry weight ($R^2 = 0.42$, $p < 0.001$, data not shown).

Soil characteristics, and their variation among sampling areas

The pH ($CaCl_2$) of the soil samples ranged from 4.0 to 6.8 with a mean of 5.5 (Table 1). The samples were generally low in total nitrogen (mean 0.23%), OC (mean 2%), EC (median 0.09 units) and available P (Bray-I, median 0.1 units). The samples had high PBI (median 279.8) indicating high P fixation potential of the soils. Clay content was generally high, with a maximum of 49.5% (mean 34.4). Soil texture ranged from clays (e.g. soils 29 and 57) to sandy clay loams (e.g. soils 19 and 30) and included

clay loam soils (e.g. soil 36) and sandy clay (e.g. soil 1).

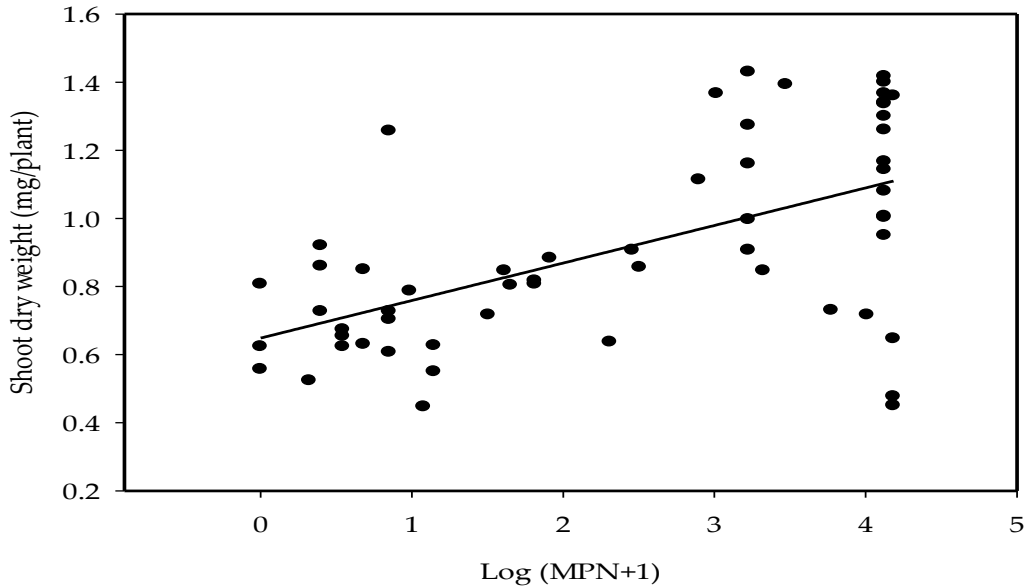


Figure 4. Regression line showing the association between rhizobial populations (cells/g soil) determined by the most probable number (MPN) technique and shoot dry weight of plants ($p < 0.001$).

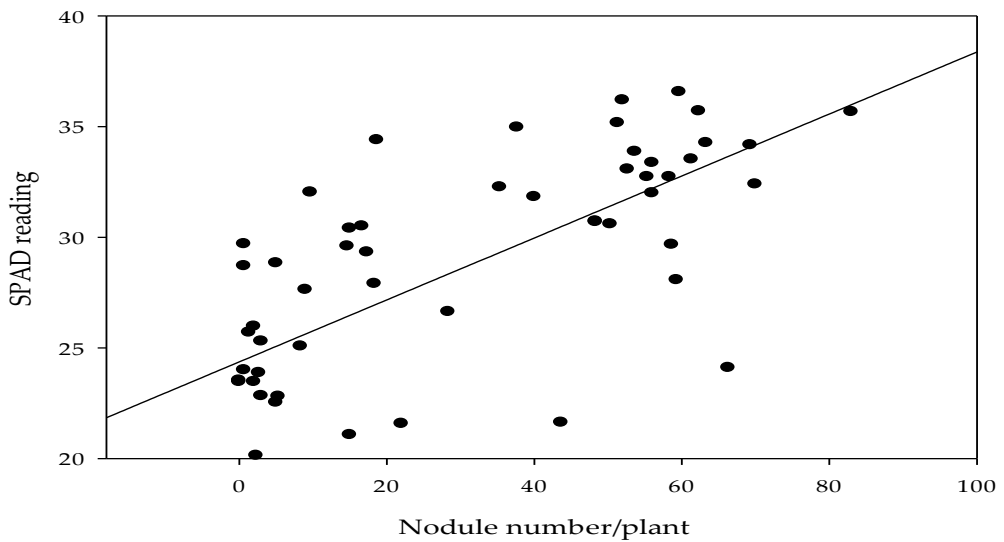


Figure 5. Regression between nodule number per plant and SPAD reading of the middle leaflet of the first trifoliate leaves 28 d after germination in the greenhouse ($p < 0.001$).

Table 1. Soil characteristics measured for 55 soil samples collected from soybean-growing areas of Ethiopia and their linear regression with rhizobial abundance and effectiveness.

Variable	Mean ($\pm$ s.e.)	Median	Min	Max	Linear regression Log (MPN+1)			Linear regression Effectiveness		
					p-value	R ²	slope	p-value	R ²	slope
pH H ₂ O	6 ($\pm$ 0.1)	6	5	8	0.1	-	-	0.9	-	-
pH CaCl ₂	6 ($\pm$ 0.1)	6	4	7	0.9	-	-	0.9	-	-
Total N%	0.2 ($\pm$ 0.01)	0.2	0.04	0.4	0.05	0.0	-0.01	0.8	-	-
Nitrate (mg/kg)	10 ($\pm$ 1.1)	7	1	30	0.2	0.0	-	0.1	-	-
Ammonium (mg/kg)	22 ($\pm$ 1.3)	23	4	35	0.7	-	-	0.5	-	-
C%	2 ($\pm$ 0.08)	2	1	3.3	0.02	0.1	-0.10	0.6	-	-
Conductivity (ds/m)	0.1 ($\pm$ 0.01)	0.1	0.03	0.2	0.6	-	-	0.5	-	-
P(Bray-I) (mg/kg)	3 ($\pm$ 0.7)	1	0.1	24	-	-	-	0.7	-	-
P(Cowell) (mg/kg)	16 ($\pm$ 1.2)	14	4	44	1.0	-	-	0.9	-	-
K (mg/kg)	449 ($\pm$ 43)	347	98	1097	0.9	-	-	0.1	-	-
PBI	280 ($\pm$ 21)	309	57	558	0.9	-	-	0.6	-	-
Clay%	34 ($\pm$ 0.9)	34	25	50	0.2	-	-	0.1	-	-
Silt%	10 ($\pm$ 0.5)	10	2	19	0.4	-	-	0.3	-	-
Sand%	55 ($\pm$ 1)	56	36	67	0.07	-	-	0.02	-	-2.3
Effectiveness	2	1	1	3	<0.001	.47	0.4			

The principal component analysis of the site and soil data showed that the first four components explained 75% of the variation among the samples. Principal component 1 (PC1) contained the major classifying factors: mineral N, P, conductivity, pH, PBI and clay content, explaining 36% of the variation, while PC2 included organic carbon and P on PC1 factors and explained 17% of the variability (Table S2). The first two major components (PC1 and PC2) grouped the soil collection sites into four quadrants that closely aligned

with their geographic locations (Figure 7). Each geographical region was associated with different soil properties; samples from SE were typically defined by the levels of P, nitrate and conductivity, whereas samples from WE samples were defined by the levels of total nitrogen and organic carbon; samples from SWE were defined by clay, symbiotic effectiveness and rhizobial population density, and those from BG by pH (Figure 6).

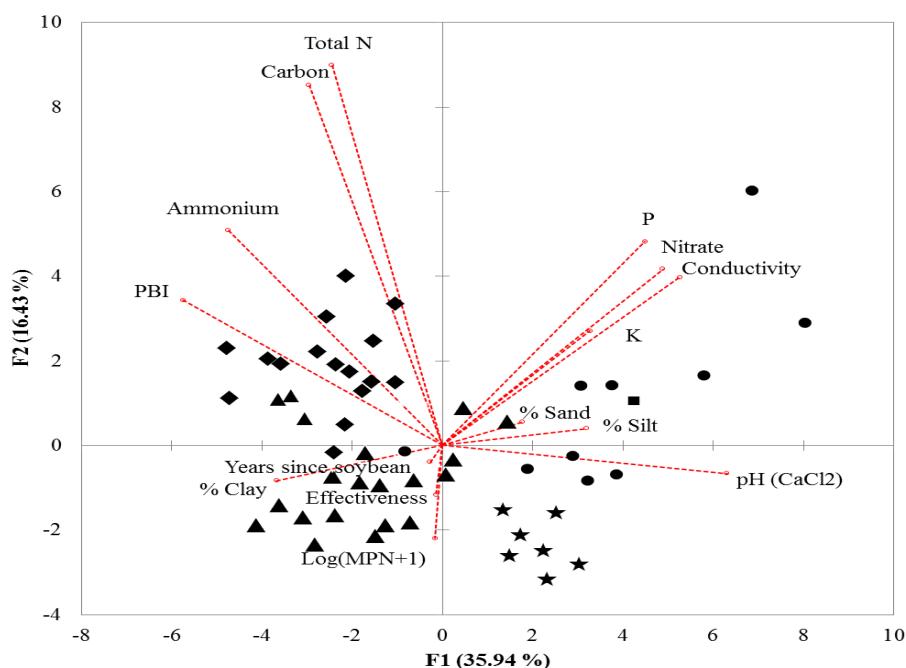


Figure 6. Plot of principal components (PCA1 and PCA2) showing grouping of sampling sites and associated soil properties dominant for these sites. Different areas of Ethiopia are indicated: Southern Ethiopia (●), Western Ethiopia (◆), Southwestern Ethiopia (▲), and Assossa (★). F1 and F2 explain 52.36% of the variation in the data.

Among the soil properties measured, organic carbon ($R^2 = 0.1$) and total nitrogen ($R^2 = 0.07$) were weakly and negatively correlated with the rhizobial populations, while no correlation was found with other soil properties. The symbiotic effectiveness of the rhizobial populations was positively correlated with MPN ($R^2 = 0.47$) and negatively correlated with the proportion of sand ($R^2 = 0.09$, Table 1).

Rhizobial density in the soil has been positively correlated with soil properties (organic matter, pH, base saturation) and environmental factors (rainfall, irradiance, and the presence and vigour of legumes) (Bottomley, 1991, Drew *et al.*, 2012). However, in this study, rhizobial density in soil was weakly and negatively correlated only with organic carbon and total nitrogen, as indicated by the principal component analysis. The reason for this observation is not clear, as rhizobia numbers have typically been associated with higher organic carbon and total nitrogen in soils (Chatel and Greenwood, 1973; Coventry *et al.*, 1985, Zhang *et al.*, 2011).

There was no correlation between rhizobial density and 11 soil variables (Table 1) across all growing areas. In a comparative study, tropical soil rhizobial populations were positively correlated with P retention and negatively with pH (Woomer *et al.*, 1988). In soils from African countries,

only pH was correlated (negatively) with soybean rhizobial numbers (Abaidoo *et al.*, 2007). Thus, while pH is often a strong driver of rhizobial numbers in soils, this was not observed in the present study, and unexpectedly large numbers of rhizobia found in acid soils might indicate some adaptation of the rhizobial populations, which might provide a source for collection of acid tolerant strains.

Conclusions

The soil population of soybean-nodulating rhizobia and their effectiveness varied among fields and areas in Ethiopia, providing both opportunities and challenges to improve soybean yield through inoculation. Among the major growing areas, soil samples from SE, WE and AZ generally contained smaller soybean-nodulating rhizobial populations (76% with < 300 cells/g soil), providing circumstances where inoculation is likely to improve the growth of soybean. Extension efforts to improve the availability and rates of inoculation would therefore be more worthwhile in these regions. Higher proportions of soil samples from SWE contained large numbers of rhizobia (95% of soils contained > 300 cells/g soil) with similar effectiveness to CB1809 (86%). Inoculation with rhizobia is therefore unlikely to improve soybean growth in the

majority of fields in SWE. Although there were limited correlations between soil rhizobial numbers and other soil and cultural (crop history) practices, higher populations in the SWE region may provide an opportunity to target strains that are well-adapted to acid soils.

Supplementary Materials: The following are available online at www.mdpi.com/link, Table S1: Soil collection areas and regions with cropping, inoculation history and rhizobial populations determined using most probable number (MPN) technique, Table S2: Normalised principal component loadings from principal component analysis of 15 soil variables.

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Supplementary material

Table S1. Soil collection areas and regions with cropping, inoculation history and rhizobial populations determined using most probable number (MPN) technique. Oromia Regional State is represented by Southwestern Ethiopia (Jimma, and Chewaka areas), and Western Ethiopia (Bako and Gute areas). Southern Ethiopia represents the Southern Nations and Nationalities People (SNNP) and extends from Hawassa to Amaro areas. Benishangul Gumz (BG) Regional State is represented by Assossa Zone (AZ).

Number	Soil code	Region	Area	Inoculation history	Years since last soybean	Log (MPN+1)
1	Jimma-03	Oromia	SWE	No	1	4.01
2	SE-32	SNNP	SE	No	4	2.31
3	Bako-03	Oromia	WE	No	6	4.18
4	Assossa-1	BG	AZ	No	1	4.18
5	Jimma-11	Oromia	SWE	No	0.4	4.18
6	SE-39	SNNP	SE	Yes	1	3.77
7	Bako-10	Oromia	WE	No	0.4	1.08
8	Assossa-2	BG	AZ	No	1	0.54
9	Jimma-24	Oromia	SWE	No	0.4	3.32
10	SE-10	SNNP	SE	No	6	0.54
11	Bako-04	Oromia	Oromia	WE	6	0.32
12	Jimma-14	Oromia	SWE	No	4	1.65
13	SE-20	SNNP	SE	No	6	2.46
14	Bako-18	Oromia	WE	No	1	0.54
15	Assossa-3	BG	AZ	No	1	1.91
16	Assossa-4	BG	AZ	No	2	0.99
17	Jimma-40	Oromia	SWE	No	2	3.47
18	Jimma-37	Oromia	SWE	No	∞^1	3.23
19	Jimma-08	Oromia	SWE	No	1	3.23
20	Jimma-05	Oromia	SWE	No	2	3.02
21	Jimma-34	Oromia	SWE	No	1	4.12
22	Jimma-27	Oromia	SWE	No	0.4	4.12
23	Jimma-25	Oromia	SWE	Yes	1	4.12
24	Jimma-16	Oromia	SWE	No	∞	4.12
25	Jimma-12	Oromia	SWE	Yes	1	4.12
26	Jimma-01	Oromia	SWE	No	1	4.12
27	Jimma3	Oromia	SWE	No	0.4	4.12
28	Jimma-10	Oromia	SWE	No	1	4.12
29	Jimma-28	Oromia	SWE	No	1	4.12
30	Jimma-09	Oromia	SWE	No	1	4.12
31	Jimma-32	Oromia	SWE	Yes	1	4.12
32	SE-33	SNNP	SE	No	3	1.81
33	SE-26	SNNP	SE	No	3	1.81
34	SE-36	SNNP	SE	No	0.4	3.23
35	SE-05	SNNP	SE	No	∞	0.85
36	SE-38	SNNP	SE	Yes	1	3.23
37	SE-37	SNNP	SE	Yes	1	1.61
38	SE-30	SNNP	SE	No	0.4	0.85
39	Bako-30	Oromia	WE	No	0.4	1.15
40	Bako-36	Oromia	WE	No	0.4	0.4
41	Bako-40	Oromia	WE	No	1	0.85
42	Bako-20	Oromia	WE	No	∞	0.4

Number	Soil code	Region	Area	Inoculation history	Years since last soybean	Log (MPN+1)
43	Bako-07	Oromia	WE	No	4	0
44	Bako-31	Oromia	WE	No	0.4	0.68
45	Bako-25	Oromia	WE	Yes	0.4	4.12
46	Bako-28	Oromia	WE	No	0.4	1.15
47	Bako6	Oromia	WE	No	0.4	4.12
48	Bako-12	Oromia	WE	No	6	0.4
49	Bako-32	Oromia	WE	Yes	0.4	0
50	Bako-22	Oromia	WE	No	1	0.68
51	Assossa-8	BG	AZ	No	4	0.85
52	Assossa-6	BG	AZ	No	1	2.51
53	Assossa-5	BG	AZ	No	3	1.51
54	Jimma-39	BG	AZ	No	1	2.9
55	Jimma-38	BG	AZ	No	1	3.23

∞¹ represents farms where soybean had never been grown

Table S2 Normalised principal component loadings from principal component analysis of 15 soil variables.

	F1	F2	F3	F4	F5	F6
Rhizobia MPN	-0.179	-0.379	-0.545	0.63	0.094	0.17
Years since soybean	-0.034	0.033	0.38	-0.183	0.756	0.321
Ammonium	-0.69	0.504	-0.226	-0.022	0.061	0.243
Nitrate	0.741	0.381	0.069	0.129	0.262	-0.22
P Colwell	0.669	0.429	-0.272	0.166	0.087	-0.071
K Colwell	0.477	0.255	-0.321	-0.217	0.017	0.665
Carbon	-0.406	0.863	0.013	0.153	-0.108	-0.019
Conductivity	0.666	0.373	-0.217	0.018	0.179	-0.411
pH CaCl ₂	0.932	-0.071	-0.036	-0.117	-0.065	0.121
pH H ₂ O	0.927	-0.014	-0.021	-0.079	-0.052	0.216
PBI	-0.846	0.321	-0.088	0.231	-0.032	-0.03
Total N	-0.331	0.9	-0.1	0.092	-0.068	0.132
P(Bray I)	0.785	0.34	-0.162	0.214	0.161	-0.14
Clay%	-0.544	-0.071	-0.499	-0.468	0.338	-0.204
Variability	35.814	16.984	12.768	9.747	6.368	6.063
Cumulative% variance	35.814	52.798	65.567	75.314	81.682	87.745

Effects of C₁ and C₃ Seed on Yield and Economic Benefits of Bread Wheat (*Triticum aestivum*. L) in Arsi and Bale Areas, Ethiopia

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Abstract

The quality seed of certified generation one (C₁) is one of the determinant factors limiting the productivity of improved varieties. In most of sub-Saharan Africa, farmers use farm-saved seed. However, very limited information is available whether certified C₁ seed is better than farm-saved seed (C₃) in increasing productivity. The objective of the study was, therefore, to evaluate the effects of C₁ and C₃ of improved varieties on yield and yield components of bread wheat (*Triticum aestivum*. L). The experiment was conducted during the 2018 cropping season at Lole, Herero, and Tamela commercial farms, which are known to be most suitable areas for bread wheat varieties to express potential productivity. Three bread wheat varieties (Hidase, Hulluka, and Ogolcho) and two seed classes (C₁ and C₃) plus hand cleaned and uncleaned seed of local check variety were arranged in 2×4 factorial combinations using randomized complete block design. Variations of testing environments, varieties, and seed generations showed significant differences in yield and yield traits. Hulluka followed by Ogolcho varieties showed the highest yield performance compared to Hidase and the local one. Combined mean reveals that the use of certified seed (C₁) over C₃ increased grain yield, ranging from 11 to 33% and mean was 18.3%. Besides, the use of C₁ could, on average, earn up to 12,117 ETB ha⁻¹ extra net returns than when C₃ was used. In conclusion, the use of C₁ of improved varieties is the best bet technology to enhance the productivity and profitability of smallholder farmers, justifying frequent seed replacement rate than usually stipulated. The use of upgraded farm-saved seeds of improved varieties is also an alternative option for smallholder farmers as significantly higher economic yield could be harvested when compared with the local check. However, it should be clear that the use of C₃ of improved varieties has at least 11% and at the utmost 33% yield penalty when compared with C₁ and the penalty could be higher for more than C₃ generation of farm-saved seeds. Therefore, the seed replacement rate of bread wheat varieties should be shorter in the Ethiopian seed system to ensure sustainable crop production.

Keywords: Bread wheat, certified seed, quality seed, seed replacement rate, land productivity

Introduction

Seed is a key input that substantially contributes to productivity increment of a given crop. It is a vehicle that delivers almost all of technological innovations to farmers so that they can exploit the genetic potential of new varieties (Shahwani *et al.*, 2014; Walsh *et al.*, 2014). Seed quality can be described in terms of four main factors (Khazaei *et al.*, 2016), which include genetic identity, physical purity, physiological quality, and seed health. Therefore, seed quality is not a single attribute, but rather comprises a number of discrete components, each of which worth consideration for increasing crop productivity. Most of the time, improved variety of a given crop is considered as quality seed. However, genetic and physical purity, physiological and health aspects of seed are of paramount importance.

Most of the Sub-Saharan African farmers use farm-saved seeds (often not of high quality) for the production of major crops, including bread wheat (*Triticum aestivum* L). In Ethiopia, depending on crop and variety, the extent of use of farm-saved seed and its quality are quite variable and such seed could be sourced from own saving or through an exchange, gift, and local grain/seed market (Abebe Atilaw 2011; Dawit *et al.*, 2017). The main reasons for use of farm-saved seed by most of the smallholder farmers of Sub-Saharan Africa are better price, availability, accessibility,

reliability and sustainability compared with seeds that could be accessed from the formal seed systems.

In general, yield penalty from use of farm-saved seed or local ones depend on the percentage of seed replacement rate (SRR), which gives an idea about the quantity of the quality seed used by smallholder farmers (Bhusal, 2017). The SRR refers to the percentage of area sown out of the total area of a crop planted in the season by using certified/quality seeds other than the farm-saved seeds. Many reports indicate that the average desirable or standard SRR, without which it is not possible to achieve higher productivity is 25% for self-pollinated crops including bread wheat, which has a strong positive correlation with yield (Singh and S.Singh, 2016). Even though there is no tangible information on SRR for different crops, it is generally very low for bread wheat compared to hybrid varieties of maize as the majority of wheat producing areas are still using farm-saved seed. For instance, SRR for bread wheat in 2017 cropping season, based on the total amount of certified seed distributed (personal communication with Ministry of Agriculture and regional agricultural office) and wheat production area (CSA, 2017) was 9.9% at country level. A similar report also indicates SRR in 2011 cropping season was 11 and 18 in Nepal and India, respectively which is below the minimum average to ensure

sustainable productivity (Bhusal, 2017). Low SRR indicates that the majority of smallholder farmers use farm-saved seeds through informal ways, which could have a significant yield penalty (Amir *et al.*, 2007; Singh and S. Singh, 2016).

In contrast, certified seed (like C_1) of new varieties offer benefits of yield increase, tolerance to biotic and abiotic stresses due to their improved genetic make-up (Krishna *et al.*, 2016). For instance, about 10-20% yield increasement of bread wheat was observed when a certified seed with a higher thousand seed weight was used compared to farm-saved seed with lower seed weight (Shahwani *et al.*, 2014) and vice versa. Similarly, Amir *et al.*, (2007) reported that about 88.6% and 44.4% yield reductions could be recorded as a result of using C_4 and C_3 generation of farm-saved seeds of improved varieties of bread wheat, respectively when compared with C_1 of the same variety. Alemayehu (2015) reported that 100% yield advantage could be attained in bread wheat when C_1 of an improved variety was used instead of farm-saved seed of a local variety in Eastern Ethiopia. In addition, many research findings reveal that seed upgrading like cleaning and grading of even farm-saved seed can significantly improve germination potential, seedling uniformity, plant stand, reduce cost and as a result increase the yield (Barnard and Calitz, 2011). However, it should be noted that the use of farm-saved seeds of even

improved varieties can have a significant yield penalty when compared with certified seed of the same variety.

Although measuring the contribution made by the use of different generations of certified seeds of improved varieties to yield increase is essential, the process remains challenging as it is not easy to attribute the contribution of quality seed to yield increase relative to other agricultural inputs and management practices. Integrated Seed Sector Development (ISSD) Ethiopia Programme has been supporting seed producer organizations, with the goal of increasing the use of quality seed of improved varieties of different crops so as to contribute to increased productivity. Most farmers have been using farm-saved seed (either of improved or local variety), particularly for self-pollinated crops like bread wheat. However, very limited information is available whether a farm-saved seed (either quality seed or not) or improved variety of seed (either quality seed or not) could increase the productivity of the crop. Therefore, the objective of this study was to evaluate the effects of C_1 and C_3 seeds of improved bread wheat varieties on productivity.

Materials and Methods

Description of experimental areas

The experiment was conducted at three seed multiplication farms i.e Lole,

Herero and Tamela of Oromia Seed Enterprise (OSE), located in Arsi and Bale zones of Oromia, Ethiopia. The farms are located in the bread wheat growing belt of the country. The areas receive mono-modal rains and the active growing period is from June to mid-September. Five-year average annual rainfalls for Lole, Tamela and Herero farm are 516, 628 and 720 mm (Figure 1), respectively. Tamela farm

had no adequate moisture after seed planting and rainfall shortage, after mid-September to October was evident in the 2018 cropping season when the crop critically needs an adequate amount of moisture for grain filling. In addition, this farm is characterized by an unexpected frost problem during the grain filling stage, mid-September to October.

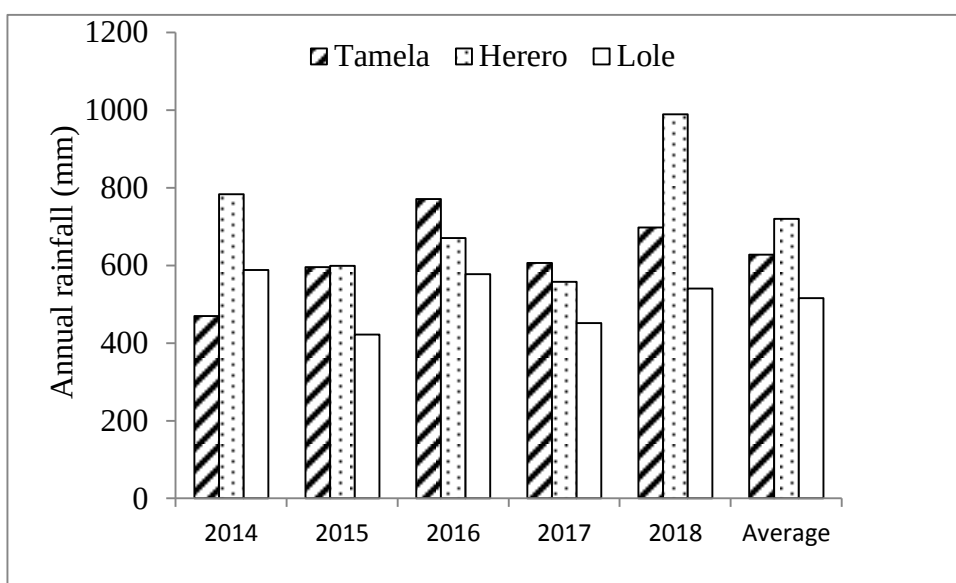


Figure 1: The annual rainfall amount (mm) of the experimental environments

Experimental materials and design

The experimental trial consisted of two factors: “Four bread wheat varieties i.e. Hidase, Hulluka, Ogolcho, and ‘local’ check (Enkoy)” and two seed classes (C_1 = certified seed and C_3 = farm-saved seed) of the same varieties. In real situation, there is no local varieties as bread wheat is not the center of origin or diversity in Ethiopia. We considered the check as ‘local’, which was named as ‘Enkoy’

(K6295-4A) since it was released in 1980 and has been used for a long period of time, without periodical variety purification/maintenance. Although the variety is currently obsolete, it is still under production by some farmers in Arsi Zone. Manually cleaned (clean is a manual separation of inert matters, undersized seed, weed seed and other unwanted materials) and unclean seeds were considered as treatment levels for the ‘local’ check since its C_1 is not produced by the seed

producers. Therefore, the first factor was four bread wheat varieties including the 'local' check whereas the second factor is the level of quality seed (C_1 and C_3) as well as cleaned and unclean seed for the local check. Eight treatment combinations for three improved varieties with two seed class levels (C_1 and C_3) plus the local check as cleaned and unclean seeds were arranged in factorial combinations using a randomized complete block design (RCBD), replicated four times.

Experimental procedures

The experimental plots were cultivated two to three times in each farm and leveled very well before planting. The gross size of each experimental plot was $1.6 \text{ m} \times 2.5 \text{ m} = 4 \text{ m}^2$ and the distance between plots and blocks were 50 cm and 1.5m, respectively. Eight rows in 20 cm spacing were prepared on each plot using locally available material. The amount of seed required for each plot, regardless of the variety and seed classes, was prepared on the basis of 125 kg ha^{-1} recommended seed rate. The seed was then planted in mid-June by drilling in the prepared rows. The same amount of fertilizer was applied for each plot based on the locally recommended rate, $100 \text{ kg NPS ha}^{-1}$ and $50 \text{ kg Urea ha}^{-1}$. The NPS fertilizer was applied in the rows at the time of planting; half of the Urea was applied at the time of planting whereas the remaining half was applied at the time of tiller initiation, 21 days after planting. All other agronomic managements were uniformly applied. Weed was

uniformly controlled by the application of recommended rates and types of herbicides. Similarly, an appropriate rate and type of fungicides were uniformly applied in order to manage yellow and stem rusts of wheat.

Data collection and analysis

Yield and yield component data were collected from the time of planting until harvesting. Days to maturity, grain yield, and 1000 seed weight were collected. In addition, absolute yield, calculated as yield obtained from C_1 of respective bread wheat varieties minus yield obtained from the same varieties of C_3 , and relative yield response, calculated as additional yield obtained due to use of C_1 of bread wheat varieties divided by yield obtained from farm-saved seed of the same respective varieties were recorded. Economic benefit, calculated as revenue gained, $14 \text{ ETB } 1 \text{ kg}^{-1}$ of grain, due to additional yield obtained from C_1 of bread wheat varieties minus relevant input cost, sum of cost of C_1 seed of bread wheat varieties and the cost of combine harvester for absolute yield obtained with an estimation of 40 ETB per one quintal, in relation to farm-saved seed due to use of C_1 compared to C_3 for each variety were calculated. Finally, the collected data were subjected to analysis of variances using SAS software to quantify the level of significance. Mean separations were done using LSD ($P=0.05$) value.

Results and Discussion

Purity and germination analysis

Purity percentage of farm-saved seed was lower than that of C_1 seed for all varieties, indicating that the quality level of farm seed was considerably lower than that of the certified seeds (Table 1). Germination analysis indicated that the germination capacity of C_3 for all varieties was inferior to

the C_1 of the same respective varieties. For instance, germination capacity of C_3 of Hidase variety was lower by 12% compared to C_1 whereas it was lower by 9% and 8% for Ogolcho and Hulluka varieties, respectively. The variations in germination capacity have a positive correlation with stand count per unit area and thereby cause yield variability (data not presented).

Table 1: Purity and germination percentage of seed classes of bread wheat varieties

Variety	Seed class	Seed quality parameters	
		Purity (%)	Germination (%)
Hidase	C_1	98	91
	C_3	95	79
Hulluka	C_1	98	91
	C_3	97	83
Ogolcho	C_1	98	89
	C_3	97	81
Local	farm-saved	95	73

The effect of seed quality on bread wheat traits

The result of days to maturity due to the use of different quality seed showed considerable variations (Figure 2). In line with other finding, use of C_1 mature earlier than C_3 though varietal difference also affected days to mature (Gadisa, 2019). For instance, C_1 of Hulluka variety was matured by a week earlier than its C_3 . Earlier crop maturity has a paramount importance, particularly to escape low moisture stress or in the case of early cessation of rainfall during grain filling stage. This was particularly observed at Tamela since the rain was

not adequate during flowering to grain filling stage (Figure 5), in spite of which significant yield increase was noted due to using C_1 compared with the use of C_3 seed. Seedling vigor was significantly higher for C_1 compared to C_3 . The findings of other studies (Mohsen *et al.*, 2011; Ambika *et al.*, 2018) showed similar results. The highest seedling vigor was recorded when C_1 of Hulluka variety was used whereas the lowest was recorded from C_3 of Ogolcho variety (Figure 3A). In general, poor quality seeds show low viability, reduced germination, low emergence rates, and poor tolerance to sub optimal conditions and low

seedling growth rates, finally resulting in low yield (Gadisa ,2019).

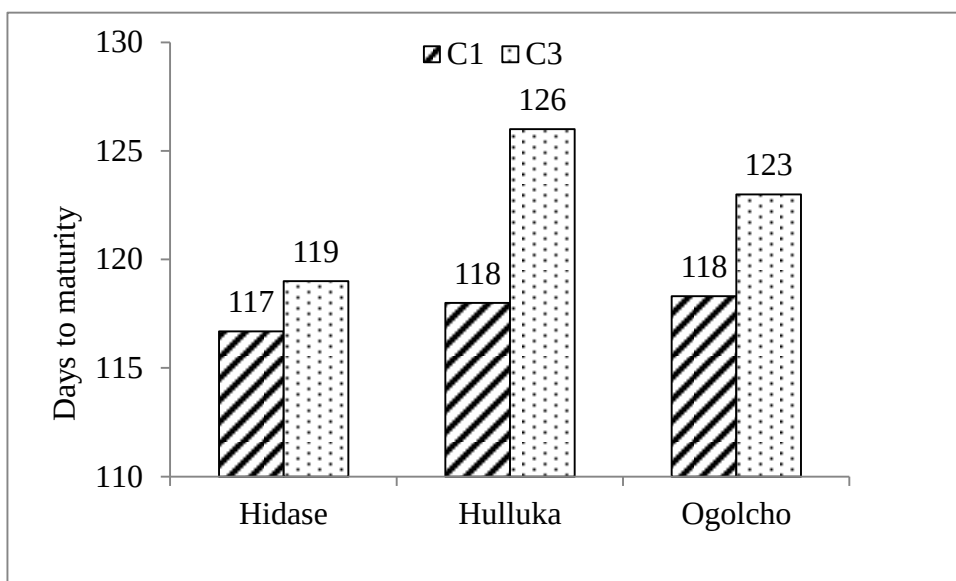


Figure 2: The effect of quality seed on maturity duration of bread wheat varieties at three environments (Lole, Tamela and Herero farms)

In addition to seedling vigor and maturity date, stand count per unit area at the time of harvesting was considerably affected by the use of different seed quality (Figure 3-B). Stand count recorded per unit area under C_1 was higher than C_3 ; the highest stand count per unit area was recorded when C_1 of Hulluka variety was used, and an increase of 12% over C_3 was evident. On the other hand, the population of variety Hidase was

significantly lower than that of other varieties even if plots under C_1 of the variety had higher stand count than its corresponding C_3 (Figure 3-B). This may be related to the seed size of the particular variety as its thousand seed weight was significantly higher than other varieties. This may indicate that crop varieties may require different recommendations of seed rate depending on their seed weight per unit count.

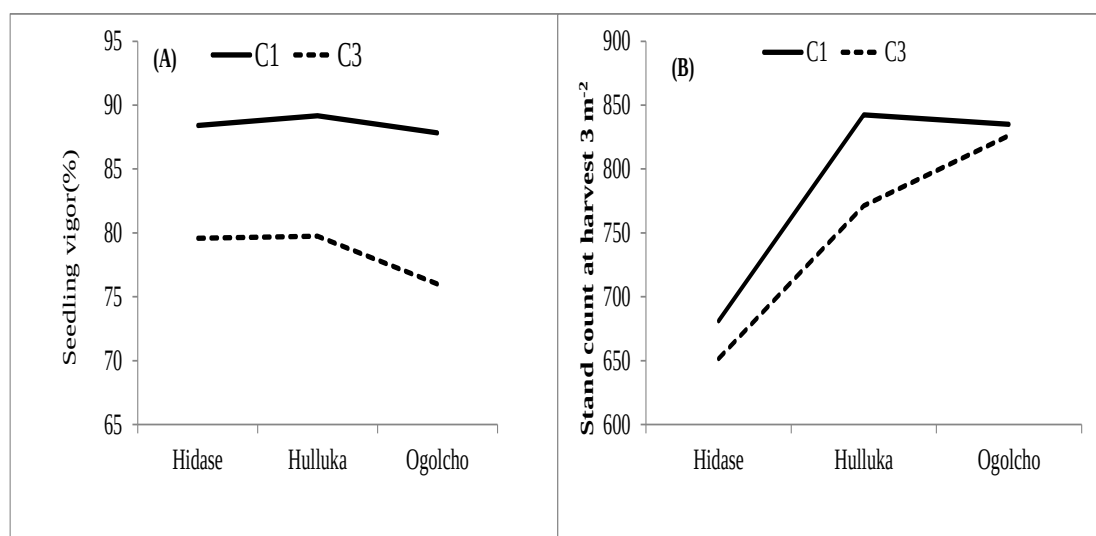


Figure 3: The effect of seed quality on seedling vigor (A) and stand count per unit area of bread wheat varieties at three environments (Lole, Tamela and Herero farms)

The effect of seed quality on yield of bread wheat

The analyses of variances indicated that the use of different seed quality resulted in significant yield variations (Table 2). In addition, environment

and varieties showed considerable variations. However, our main interest is to deal with the differential effect of certified seed versus farm-saved seed of the crop.

Table 2: Analysis of variances indicating on yield of bread wheat varieties as affected by different seed quality levels for each and combined over environments

Sources of variations	Df	Mean squares			
		Herero	Lole	Tamela	Combined
Var	3	19761259.5***	2839963.9***	9161980.7***	9490395.7**
Qual	1	2465269.7**	2784107.1**	6268140***	26344657.39*
Var*Qual	3	129022.2 ^{NS}	175405.3 ^{NS}	983792.9**	352272.8 ^{NS}
Env*Qual	2	-	-	-	467973.7 ^{NS}
Env*Var*Qual	6	-	-	-	777092.6 ^{NS}
MSE	21	240715	195949	240768	-
	63	-	-	-	225810.8
CV (%)		11.13	9.58	13.67	11.3

Note: Var= Variety, Qual= Quality level of seed class, Rep= Replications, MSE= Mean square error, Df= Degree of freedom, CV=coefficient of variation

Generally higher grain yield was obtained for C₁ than C₃ seed at Tamela farm. The highest and significant yield

increase of 49% and 43% were observed when C₁ of Hidase and Ogolcho varieties were used instead of

C₃, respectively. On the other hand, the use of either C₁ or C₃ of Hulluka variety did not show significant mean yield differences (Figure 4). The response of quality seed and different varieties to stress conditions showed

significant yield variability due to higher seedling vigor and growth rate than farm-saved seeds (Moshatati and Gharineh, 2012).

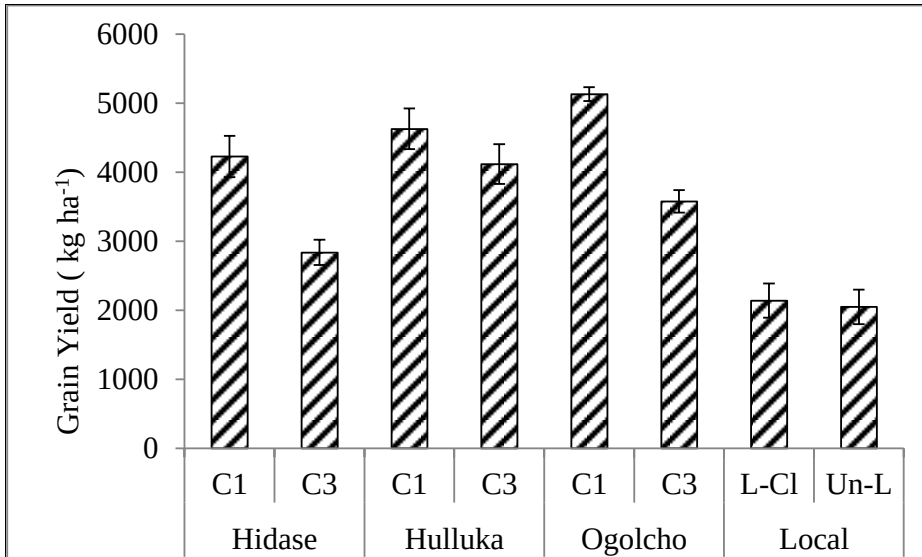


Figure 4: The effect of different seed quality on mean yield of bread wheat varieties at Tamela farm. Note: C₁= certified seed, C₃= farm-saved seed of third generation of certified, C-L= cleaned seed of local variety, Un-L= unclean seed of local variety

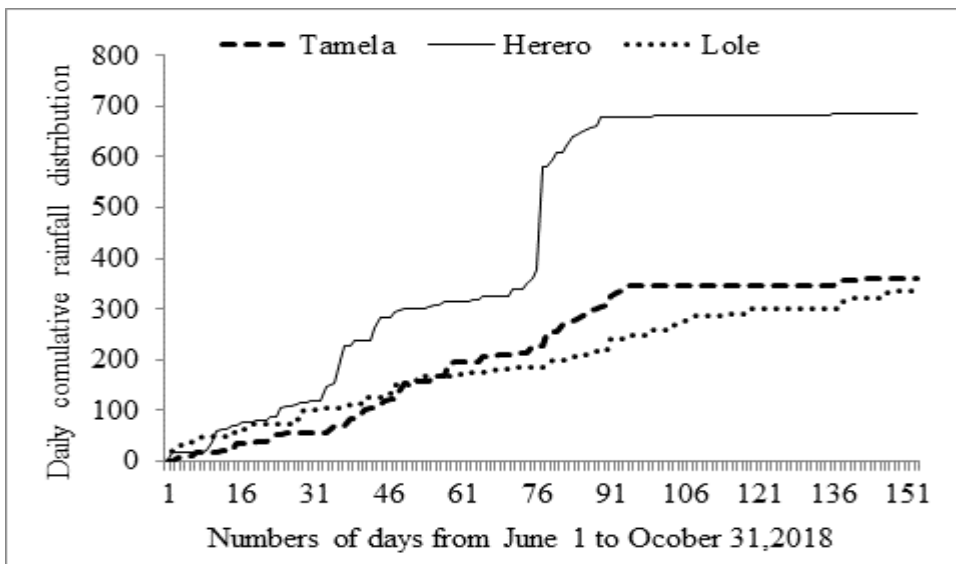


Figure 5: Daily cumulative rainfall distribution from June 1 to October 31, 2018, for each testing environment

A significant yield increase of C_1 over C_3 has happened at Tamela as there was little rainfall from the time of seed planting up to two weeks after planting, which might have a negative impact on the germination capacity of different seed quality classes (Figure 3). For instance, the germination capacity of C_1 of Hidase and Ogolcho varieties was relatively higher under such low moisture stress conditions and hence higher yield performance was recorded (Figure 3 & 4).

The results of mean grain yield indicated that the use of certified seed (C_1) significantly out-yielded at each environment when compared to the farm-saved seed (C_3) of the same varieties (Figure 6). The combined mean yield increase due to the use of C_1 over C_3 was 18.3% whereas the percentage of yield increase across the testing environments varied from 11 to 33% (Table 3). This results of this study is in agreement with other similar research finding (Mohsen 2011; Krishna *et al.*, 2016; Ambika, 2018) indicating that the use of quality seed, including seed size, increases yield by up to 15-20% compared to farm-saved seed and that the extent of yield increase is directly proportional to the use of quality seed. An increased productivity due to the use of C_1 also implies the importance of SRR to ensure sustainable productivity per unit area.

Many research findings indicate that the average SRR of bread wheat that could sustainably ensure the productivity is about 25% (Mohan *et al.*, 2013; Singh and S.Singh 2016; Ashamo and Goa, 2017). However, the SRR particularly for the self-pollinated crops in Ethiopia is low in percentage, which may account for the significant yield penalty every production year. The result at Tamela farm revealed that the use of C_1 seed, regardless of varieties, showed about 33% yield increase when compared with the farm-saved seed (C_3) of the same varieties (Figure 6 and Table 3). Tamela farm was characterized by the occurrence of inadequate soil moisture just after planting, rust disease pressure, occurrence of frost and finally shortage of rainfall at the time of grain filling stage (Figure 1 & 5). On the other hand, more than 11% yield increase was attained both at Heraro and Lole farms due to the use of C_1 over C_3 seed (Figure 6). The rainfall amount and distribution, as well as soil types and fertility status are more or less similar for both environments (Figure 1 & 5). Similar results of increase in grain yield ranging from 2.85% – 33.8% was reported in Pakistan for bread wheat as a result of using certified seed of improved varieties (Krishna *et al.*, 2016).

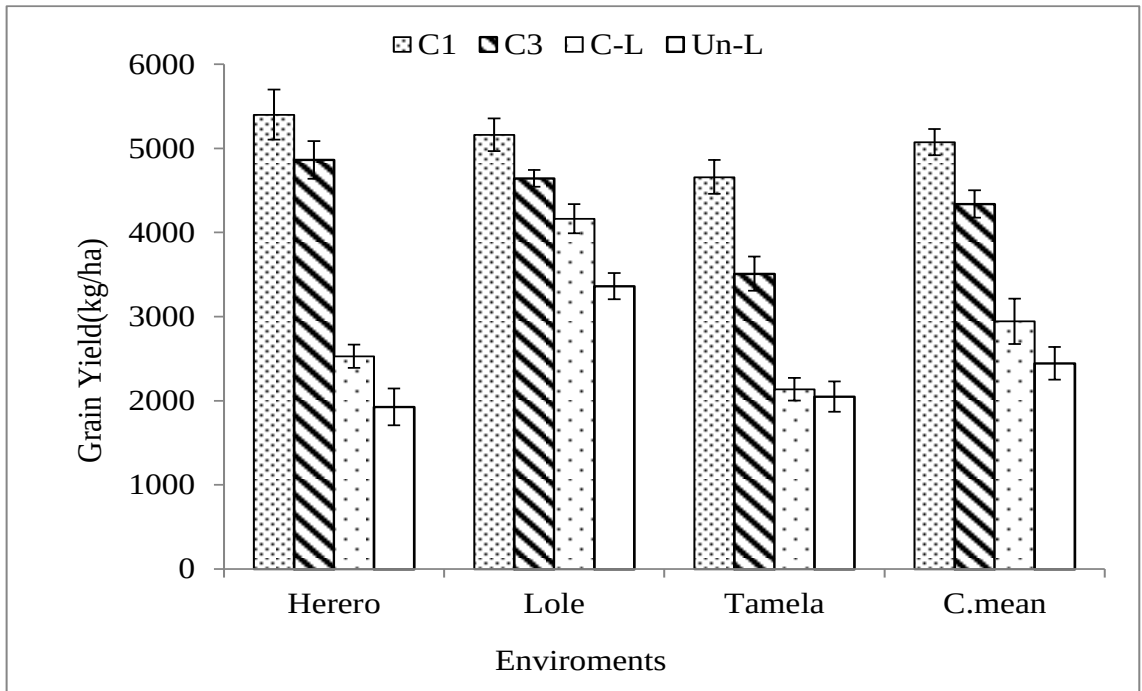


Figure 6: The effect of seed quality levels on yield of bread wheat at each testing site and combined means. Note: C1= certified seed, C3= farm-saved seed of third generation of certified, C-L= cleaned seed of local variety, Un-L= unclean seed of local variety

Table 3: Percentage (%) of grain yield increases due to uses C₁ and clean seed of local seed compared to farm-saved seed (C₃) and uncleaned seed of the local seed, and C₃ compared to local check

Seed quality	Herero	Tamela	Lole	Mean
C ₁ vs C ₃	11	33	11	18.3
Local-clean vs unclean	31.3	4.4	23.9	19.9
Mean	21.2	18.7	17.45	19.1
Farm-saved seed of improved variety versus local check used as either clean or unclean seed				
C ₃ vs local clean	92	11	64	47
C ₃ vs local unclean	152	38	71	77

The results of the current study show that the use of farm-saved seed of improved variety could be an option as it enhances yield increase of 47% to 77% compared to clean and unclean seed of local variety, respectively and this is in agreement with the findings of similar studies (Barnard and Calitz,

2011). This indicates that farmers can use their farm-saved seeds of improved varieties for some years (2-3 years) in case of shortage or limited access to C₁ of improved varieties by upgrading its quality through clearing and probably by grading (Barnard and Calitz; 2011). On the other hand, use

of cleaned seed of even local variety showed better yield advantage when compared with uncleaned one (Figure 6). The results indicate that significantly higher yield increase was observed at Herero and Lole farms due to the use of clean seed compared to unclean, and the yield increase was 31.3% and 23.9%, respectively (Table 4). This clearly indicates that seed cleaning is the most important parameters to improve its quality, particularly ensure bigger and uniform seed size that is directly proportional to uniform germination capacity (Mohsen *et al.*, 2011; Barnard and Calitz; 2011)

Absolute and relative yield response

An overall average of absolute and relative yield responses due to the use of C₁ compared to C₃ seed were 870 kg ha⁻¹ and 22%, respectively. However, the responses were different across the testing environment and the type of quality seed used (Table 4). For instance, the highest absolute yield response (1150 kg ha⁻¹) and relative yield response (36%) were recorded at Tamela farm when C₁ was used compared to C₃, indicating that the use of certified seed of improved varieties can guarantee and increase return even under stressful conditions. However, the absolute and relative yield response considerably varied in the range of 601-1150 kg ha⁻¹ and 13-

36.7% when C₁ was sown compared to C₃ seeds.

Relative economic benefit

The overall mean of the result indicated that the use of C₁ can earn up to 12,117 ETB ha⁻¹ additional net returns when compared with the use of C₃ seed. However, the net return generally varied in the range of 8318 to 16077 ETB ha⁻¹ across the testing environments, and the highest additional net return was obtained at Tamela farm as C₁ of the improved varieties withstand stressful conditions of the season such as low moisture stress and rusts to give significantly higher yield. Krishna *et al.*(2016) also reported that the highest marginal return could be obtained from C₁ of improved bread wheat varieties as compared to higher seed classes. In addition, cleaned seed of local variety significantly showed higher net returns (8808 ETB ha⁻¹) when compared with an unclean seed. This indicates that improving the quality of farmers' seeds preferably improved varieties of C₃ seed noticeably enhancing the productivity. However, it should be clear that sowing certified seeds of improved varieties principally increases the productivity of the crop and thus significantly higher net returns could be obtained when compared with C₃.

Table 4: Absolute, relative yield responses and economic benefit obtained due to the use of C₁ seeds relative to C₃

Environment	Seed class	AYR (kg ha ⁻¹)	RYP (%)	EB (ETB ha ⁻¹)
Herero	C ₁ Vs C ₃	858	15.9	11955
	Local (C ₁ vs Uncl)	547	29.5	7639
	Average	780	19.3	9797
	C ₁ Vs C ₃	601	13.3	8318
Lole	Local (C ₁ vs Uncl)	1195	33.9	16785
	Average	719	17.4	12552
	C ₁ Vs C ₃	1150	36.7	16077
Tamela	Local (C ₁ vs Uncl)	149	8.9	1999
	Average	719	22.8	9038
	C ₁ vs C ₃	870	22.0	12117
Average	Local (C ₁ vs Uncl)	630	24.1	8808
	Overall average	750	23.0	10463

Note: C₁= certified seed, C₃= farm-saved, C_i= clean seed, Uncl= unclean seed, AYR= Absolute yield response, RYP= Relative yield response; EB= Economic benefit ETB= Ethiopian Birr; Vs= versus

Conclusion and Recommendation

Certified seed of improved varieties offers benefits of yield increase and incur net profit, while farm-saved seed incurs a significant yield penalty. Most of the bread wheat producers use farm-saved seeds and low SRR and the same is true in Ethiopia. However, uses of quality seed of improved varieties has the potential to withstand stressful environmental conditions and significantly increase yield productivity per unit area. The use of C₁ can give about 18% extra yield gain on an average or at least positive absolute yield gain (870 kg ha⁻¹) when compared with C₃. Certified seed can also withstand stress condition as it has higher germination capacity, faster growth and early maturity. The use of C₁ can guarantee significantly higher yield and at least 10,000 ETB ha⁻¹ of

relative economic benefit as compared to the use of C₃ seed. In conclusion, use of certified seed of improved varieties of either general or specific adaptable varieties are the best bet technologies to enhance the productivity and profitability of smallholder farmers, but need yearly based seed replacement and purchasing ability of smallholder farmers. Therefore, many efforts are needed to increase the SRR to ensure sustainable productivity. On the other hand, uses of upgraded (through cleaning and grading) farm-saved seeds of improved varieties (e.g. C₃), particularly for self-pollinated crop varieties, for a few years (2-3 years) also offer alternative options for smallholder farmers, who are unable to access certified seed regularly or cannot afford to purchase annually. However, it should be noted that the use of C₃ seed of improved varieties can often result in yield penalty when

compared with the use of C_1 . The result of the study witness that seed replacement rate of bread wheat varieties should be shorter in the Ethiopian seed system to ensure sustainable crop productivity.

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Rainfall Variability and its Implications for Wheat and Barley Production in Central Ethiopia

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Abstract

Climate constitutes a complex set of interrelated elements and its variability induces climate shocks; thus, leading to crop failures. The objective of this study was to analyze rainfall variability and extremes and investigate the effects on wheat and barley productivity at selected sites in Central Ethiopia. Standardized rainfall anomaly index, rainfall concentration index, coefficient of variation and rainfall extreme indices were used for the historical (1980-2016) observed daily data collected from 10 meteorological stations in Central Ethiopia. Bivariate correlation/regression analysis was used to evaluate the effect of climatic variables on cereal production. The results showed that mean onset dates ranged from day of year (DOY) 157 (June 6) to DOY 181 (June 30) in the study region. On the other hand, the dates for the cessation of the seasonal rainfall ranged between DOY 271 (September 28) to DOY 288 (October 15). The annual rainfall concentration index was highly variable for most of the stations and indicates high rainfall concentration in a few months. Standardized rainfall anomaly index (SAI) showed the occurrence of moderate to severe droughts in the study region. Out of the 37 years considered for this study, 38% of the years were non-drought years while 62% of the years caused some level of drought in the region. Although no significant rainfall extremes trends were observed at most of the stations, three or more rainfall extreme indices showed a significant ($P < 0.05$) decreasing trend at Alemketema and a significant increasing trend at Fitcha, Gebreguracha and Gohatsion. Seasonal rainfall amount had a significant ($P < 0.05$) positive correlation with the yield of wheat and barley at Debre Berhan while it had a significant ($P < 0.01$) negative correlation at Holeta and Ambo. Seasonal rainfall amount explained 21-51% and 35- 65% of the variability in wheat and barley yields, respectively in Central Ethiopia. The yield of wheat and barley cultivars also showed a significant ($P < 0.05$) correlation with mean maximum and mean minimum temperatures although the level of correlation varied among sites and the cultivars studied. It is concluded that understanding the relationship between crop productivity and climate parameters is an important step towards identifying major productivity constraints and designing management and technological innovations that can address current and future climate challenges at a local level.

Keywords: Climate variability, climate extremes, cereals, crop production, central Ethiopia

Introduction

Climate constitutes a complex set of interrelated elements whereby a slight change in one variable could easily trigger uneven changes in most other elements (Alemu, 2016). This change will affect agricultural activities, which are the source of all food items consumed by humans and domesticated animals. Like any productive activity, agricultural production exerts an environmental cost. The sector contributes to the increasing climate change impacts on environments and natural resources, such as land, air, water, and biodiversity. At the same time, the sector is increasingly constrained by increasing pressure on natural resources and the growing competition with other sectors for resources (Yamusa *et al.*, 2015). Recent increases in climate variability and associated risk in agricultural practices has generated great interest in detail understanding of climate events at local level, while aiming at possible solutions.

The risk associated with climate variability poses a direct impact from the start of land preparation to final harvest (Akinseye *et al.*, 2013; Mesike & Esekhade, 2014). The characteristics (e.g., amount, temporal distribution and geographical coverage) of rainfall during the main rainy season are the most important determinants of agricultural activity. The erratic rainfall patterns, including onset and cessation dates and

distribution of rainfall govern crop yields and determines the choice of the crops to grow (Getachew and Tesfaye, 2015), thus requiring appropriate management. Belay (2010) reported that rainfall variability directly or indirectly affects agricultural production by influencing the growth and development of vegetation, emergence and distribution of crop pests, livestock diseases, aggravating the frequency and distribution of adverse weather conditions, reducing water availability, aggravating the frequency and distribution of adverse weather conditions, reducing water supplies and enhancing the severity of soil erosion. While rainfall variability has always been a major challenge for Ethiopian agriculture, *kiremt* rainfall is less variable in most parts of the country compared to the *belg* season (Bewket & Conway, 2007; Ayalew *et al.*, 2012; Mengistu *et al.*, 2014). *Belg* rainfall is highly variable and its impact on agriculture is higher due to variable onset (Abebe, 2006).

An increase or a decrease in temperature may have a significant effect on the growth and yield of crops (Integrated and Africa, 2013). Higher temperatures increase evaporation and transpiration, which could reduce soil water availability and crop yield. Different scholars have shown the importance of rainfall variability on crop production at different spatial scales globally (Jones & Thornton, 2003; Rowhani *et al.*, 2011; Adamgbe & Ujoh, 2013; Ajayi *et al.*, 2013; Afifi *et al.*, 2014; IPCC, 2014; Mwaura & Okoboi, 2014; Ray *et al.*, 2015;

Yamusa *et al.*, 2015). In Ethiopia, a few studies that show the effects of climate variability on crop production (Adugna, 2005; Rosell & Holmer, 2007; Bewket, 2009; Teka *et al.*, 2012; Getachew, 2013; Kassie *et al.*, 2014; Yohannes, 2016; Abera *et al.*, 2018; Wang, *et al.*, 2018; Lalego *et al.*, 2019). However, there is no specific study conducted on the response wheat and barley crops to climate variability in Central Ethiopia. The objective of the study was, therefore, to analyze rainfall variability and extremes and investigate the effects on wheat and barley productivity in Central Ethiopia.

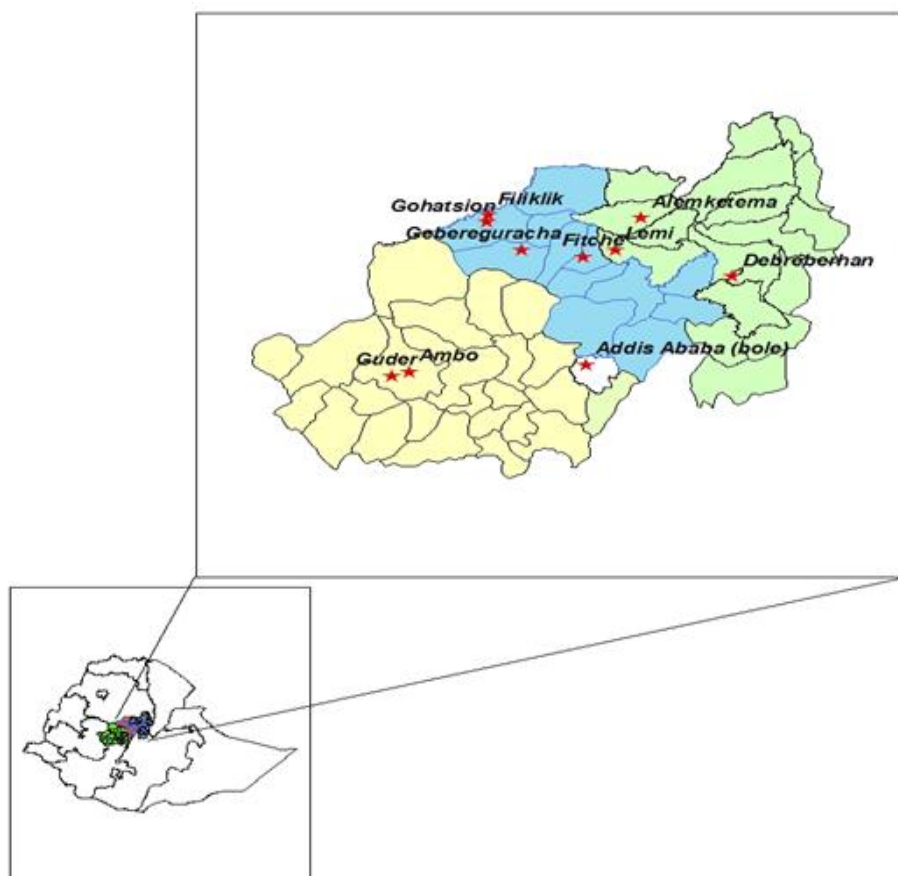
Materials and Methods

Description of the study area and data sources

The study was conducted in three administrative zones in Central Ethiopia: North Shewa Zones in Amhara and Ormia Regional States, and West Shewa Zones Oromia (Fig. 1). North Shewa Zone of the Amhara Region is located between 8° 37' 37'' to 10° 45' 14'' E and 38° 40' 13'' to 40° 10' 8'' N at an altitudinal range of 606 to 3831 m. The area receives an average annual rainfall of 920 mm with average minimum and maximum temperature of 2.4 and 23.3°C, respectively. Likewise, North Shewa Zone of Oromia Region is located between 8° 48' 32'' to 10° 23' 57'' E and 37° 30' 27'' to 39° 30' 27'' N at an altitudinal range of 880 to 3554 m; having an average annual rainfall of

1200 mm and average minimum and maximum temperatures of 6 and 21°C, respectively. West Shewa Zone is located between 8° 11' 5'' to 9° 50' 42'' E and 37° 4' 22'' to 38° 49' 25'' N with an altitudinal range of 916 to 3602 m. The area receives an average annual rainfall of 1100 mm with average minimum and maximum temperature of 11 and 26°C, respectively.

Ten study locations were selected based on agro-ecological representativeness and availability of long-term climate data. The historical (1980-2016) daily climate data of rainfall and temperatures were obtained from the National Meteorological Agency (NMA) of Ethiopia. Data were captured into Microsoft Excel version 2013 following the day-of-year entry format. In order to make the series acquiescent to further analyses, the missing data were filled using a first order Markov-chain model provided with INSTAT+v3. 37 software. The INSTAT+ v3. 37 software was also used to analyze the start and cessation of rainfall, length of growing period and dry spells. Time series data of wheat and barley yield were obtained from a crop model simulation using the Decision Support System for Agrotechnology Transfer (DSSAT) model (Hoogenboom *et al.*, 2014) conditions for the period 1984 to 2014 at Debere Berhan, Fitcha, Holeta and Ambo stations. The model was calibrated using experimental data



collected over two years at the study locations.

Figure 1. Map showing the location of ten meteorological stations used for the study in Central Ethiopia.

Analysis of onset and cessation of rainfall, probability of dry spells and length of growing period

Different authors use different threshold values to determine the onset and cessation of the rain. For this study, the definition previously used in a study in Ethiopia was adopted (Tesfaye & Walker, 2004). The onset

of rain is defined as the first occasions after 1st April when the rainfall accumulates for three consecutive days is at least 20 mm and no dry spell of more than 10 days in the next 30 days. The onset of wet and dry spells was analyzed using a first ordered Markov-chain model fitted to get more than 36 years of data for each station using the INSTAT+v3.37. The daily rainfall data was processed to give maximum dry spell lengths in the next 30 days periods, starting from the onset of date

determined for each station. The probability of occurrence of dry spells and wet spells were assessed based on both conditions that previous day was dry or wet (Stern *et al.*, 2006; Stern & Cooper, 2011). The probabilities of the maximum dry spell lengths exceeding 5, 7, 10, and 15 days over the next 30 days from the selected planting date were calculated to get a complete picture at the study areas for the whole year. Dry spells lengths of 5 to 15 days were selected in order to accommodate both drought sensitive and drought tolerant cultivars during the growing season. The cessations of the rainy season (defined as the occurrence of a day after first September when the soil water drops to 10 mm m⁻¹) was

determined using the water balance module in the INSTAT climatic guide (Stern *et al.*, 2006). Subsequently, the length of growing period in the study areas was determined as the difference between the onset and cessation dates (Tesfaye & Walker, 2004; Stern *et al.*, 2006).

Analysis of rainfall variability

Rainfall variability was examined on both annual and seasonal basis using standardized rainfall anomaly index (SAI), precipitation concentration index (PCI) and coefficient of variation (CV). The SAI index was computed as follows (Agnew, 2000);

$$SAI = \frac{(Pt - Pm)}{\sigma} \quad (1)$$

Where SAI = standardized rainfall anomaly, Pt = annual rainfall in year t, Pm = is long-term mean annual rainfall over a period of observation and σ = standard deviation of annual rainfall over the period of observation.

The drought severity classes based on SAI are: extreme drought (SAI < -1.65), severe drought (-1.28 > SAI > -1.65), moderate drought (-0.84 > SAI > -1.28) and no drought (SAI > -0.84). The PCI for annual rainfall was calculated using the formula given below (De Luis *et al.*, 2011):

$$PCI = \frac{100}{12} * [\sum_{i=1}^{12} P_i^2 / (\sum_{i=1}^{12} P_i)^2] \quad (2)$$

Following Valli *et al.* (2013) equation, the seasonal PCI for the different seasons were calculated as follows;

$$PCI = \frac{100}{3} * [\sum_{i=1}^4 P_i^2 / (\sum_{i=1}^4 P_i)^2] \text{ for the Belg season and} \quad (3)$$

$$PCI = \frac{100}{4} * [\sum_{i=1}^3 P_i^2 / (\sum_{i=1}^3 P_i)^2] \text{ for the Kirmet season} \quad (4)$$

where P_i is the rainfall amount of the i^{th} month and Σ = summation over the number of months included in the calculation.

Precipitation concentration index values of less than 10 represents a uniform rainfall distribution (i.e., low rainfall concentration); PCI values from 11 to 15 denote a moderate precipitation concentration; values from 16 to 20 denote irregular distribution and values above 20 represent a strong irregularity, i.e., concentration of rainfall in a few months of the year (Oliver, 1980).

In order to evaluate the temporal and spatial variability of rainfall, the coefficient of variation (CV) was calculated as follows:

$$CV (\%) = \frac{S}{\mu} * 100 \quad (5)$$

where S is the mean total rainfall and μ is the standard deviation.

Analysis of rainfall extremes

The rainfall indices listed in Table 1 were calculated from the daily data set for the period 1980-2016 for sites selected for the study as per the definition and calculation procedures provided by the Expert Team on Climate Change Detection Indices (ETCCDI) http://etccdi.pacificclimate.org/list_27_indices.shtml.

Table 1. Definitions of selected extreme rainfall indices

No	Index	Index name	Definition of the index	Unit
1	Rx1DAY	Maximum one day rainfall	Highest rainfall amount in one day	mm
2	Rx5DAY	Maximum five days Rainfall	Highest rainfall amount in five days	mm
3	SDII	Simple daily intensity index	Mean rainfall amount in wet days	mm/day
4	R10mm	Heavy rainfall days	Annual count of days when daily rainfall ≥ 10	Days
5	R25mm	Extremely heavy rainfall days	Annual count of days when daily rainfall ≥ 25	Days
6	R95p	Rainfall on very wet days	Annual total rainfall when daily rainfall $> 95p$	mm
7	R99p	Rainfall on extremely wet days	Annual total rainfall when daily rainfall $> 99p$	mm
8	CDD	Consecutive dry days	Maximum length of dry spell in a year (RF < 1 mm)	Days
9	CWD	Consecutive wet days	Maximum length of wet spell in a year (RF ≥ 1 mm)	Days
10	PRCPTOT	Annual total wet-day rainfall	Total rainfall in wet days (Rainfall > 1 mm)	Days

Relationship between climate variables and crops yield

Barley and wheat grain yields data were obtained from crop simulations at four sites (Debere Berhan, Fitcha, Holeta and Ambo) for the period 1984-2014 in Central Ethiopia. The yields were simulated using barley and wheat models in the Decision Support System for Agrotechnology Transfer (DSSAT), version 4.6 (Hoogenboom *et al.*, 2014). Required model inputs such as daily climate and soil parameters were collected for the respective sites. Barley (var. Cross

41/98, HB1307 and Shegie) and bread wheat (var. Alidoro, Picaflor and Hidase) varieties commonly grown by farmers and associated farmer crop management practices were used for the simulation.

The effect of climatic variables (seasonal rainfall and temperatures) on wheat and barley yield were determined using bivariate correlation and multiple linear regression analyses. The regression model for wheat and barley yield in a given zone was calculated as:

$$Y_{wt} = a + b_1x_{1wt} + b_2x_{2wt} + b_3x_{3wt} + \dots + b_nx_{nwt} \quad (6)$$

where Y_{wt} = the value of the dependent variable (yield) at station in year t ; a = Y -intercept; $b_1, b_2, b_3, \dots, b_n$ = regression coefficients; $x_1, x_2, x_3, \dots, x_n$ = independent variables (temperatures and rainfall).

Various authors (e.g. Lemi, 2005; Bewket, 2009; Rowhani *et al.*, 2011; Tunde *et al.*, 2011; Adamgbe & Ujoh, 2013; Akinseye *et al.*, 2013) used the same methodology to determine the relationship between climate variables and crop production.

Results and Discussion

Annual and seasonal rainfall amount and variability

The annual and seasonal rainfall amounts of the ten stations studied over the 1980-2016 period is presented in Fig. 2. The results showed considerable variations in annual and season rainfall amounts across the studied stations. The highest mean annual and seasonal rainfall was observed at Guder while the lowest was at Debere Berhan. Similarly, the

highest rainfall in the *kiremt* season was recorded Guder followed by Gebereguracha and Lemi stations. The *Kiremt* seasonal rainfall comprises the largest share (68-78%) to the annual total rainfall amount as compared to the *Belg* season which contributes between 15 and 23%. As observed by Game & Korecha (2015) previously, this is the typical features of the rainfall distribution in many part of Ethiopia. The annual rainfall at Filiklik, Werejarso, Gerbeguracha, and Fitcha was highly variable from year to year over the last thirty years.

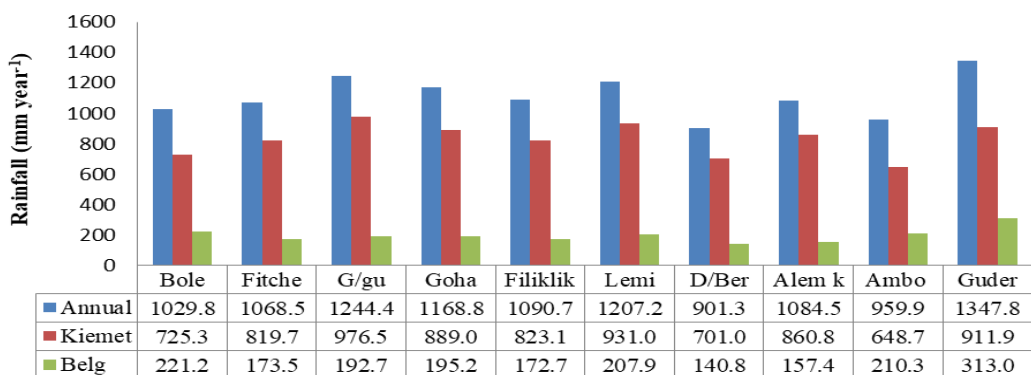


Figure 2. Annual and seasonal rainfall amounts at selected stations in central Ethiopia for the period 1980-2016.

Seasonal and inter-seasonal rainfall variability is presented in Figure 3. The CVs in the *Kiremt* season ranged from 14.7% (Gohatsion) to 33.5% (Guder) while the CVs for the annual rainfall ranged from 15% (Gohatsion) to 35.9% (Lemi). Compared to the *Kiremt* season, the *Belg* season had the highest CV which ranged from 40% (Debere Berhan) to 76% (Filiklik).

Since the *Belg* season rainfall is highly variable, it is not commonly used for growing of crops in most areas of the central highlands except in some areas in North Shewa. However, the *Belg* season rainfall is highly needed for land preparation in the central highlands, besides its role for growing of feed crops for livestock.

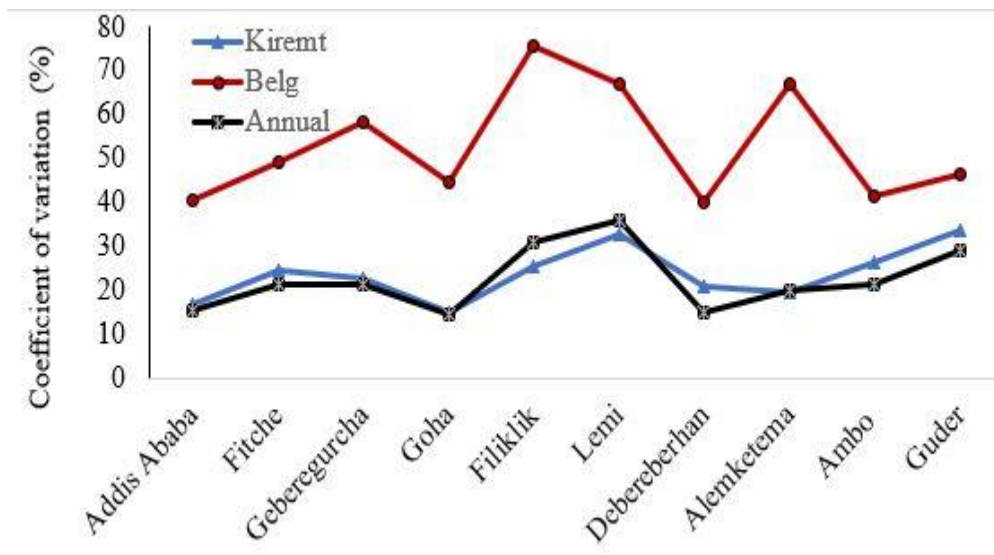


Figure 3. Annual and seasonal rainfall variability in the central highlands of Ethiopia for the period 1980-2016.

Rainfall onset and cessation dates, length of growing period and dry spells

The summary statistics for onset and cessation dates of *Kiremt* rainfall for the studied stations are presented in Fig. 4a and b. The results showed that the onset of rainfall was not symmetrical about the median indicating that the onset of the rainy season had undergone a little shift at

most of the study stations. Relative to DOY 170 (June 19), which is taken as the date when long rains used to start in Central Ethiopia, the earliest onset date over the study period was DOY 153 or June 2 (at almost all stations) while the latest onset date was DOY 218 or August 6 (Fitcha). The median onset date ranged from DOY 154 (June 3) at Guder to DOY 183 (July 2) at Fitcha (Fig. 4a) with a regional median value of DOY 169 (June 18). This indicates that the planting window in the central highlands has a

short window (from the first to second week of June) indicating a low risk of rainfall onset in the region, particularly the eastern, north eastern and central Rift Valley of Ethiopia (e.g., Hadgu *et al.*, 2017; Getachew and Tesfaye, 2017; Kassie *et al.*, 2013). The shorter planting window in the Central Highlands demand mobilization of labor and other agricultural inputs within a short period of time.

The average *Kiremt* rainfall cessation date ranged from DOY 306 (November 2) at Filiklik to DOY 366 (December 31) at Bole (Addis Ababa). The median cessation date ranged from DOY 334 (November 30) at Debre Berhan to DOY 347 (December 13) at Guder (Fig. 4b). The results show little variation in cessation date among the studied stations indicating that the cessation date have a shorter window in the central highlands.

The median length of growing period (LGP) ranged from 156 days at Fitcha to 191 days at Guder (Fig. 3c) with a regional value of 169 days. The LGP was more variable than either SOS or EOS with CV values ranging between

7% at Guder and 15% at Debre Berhan. The results indicate that the central highlands experience relatively longer growing period (more than five months) which can support long maturing crops, particularly cool season crops such as barley and wheat.

Analysis of dry spell risks

The annual dry spell analysis showed that the probability of short and long dry spells was very low once the season sets-in, mostly starting from June onwards (Fig. 5). However, the probability of dry spells increases after September and even become longer after the end of September at all of the sites studied. Compared to previous studies in the North (Hadgu *et al.*, 2013), North West (Ayalew *et al.*, 2012) and the Central Rift Valley of Ethiopia (Getachew and Tesfaye, 2015), the current results indicate very low risk of dry spells in the central highlands once the season starts. This justifies the reason why drought sensitive crops such as barley, wheat and potato are the dominant crops in the central highlands of Ethiopia.

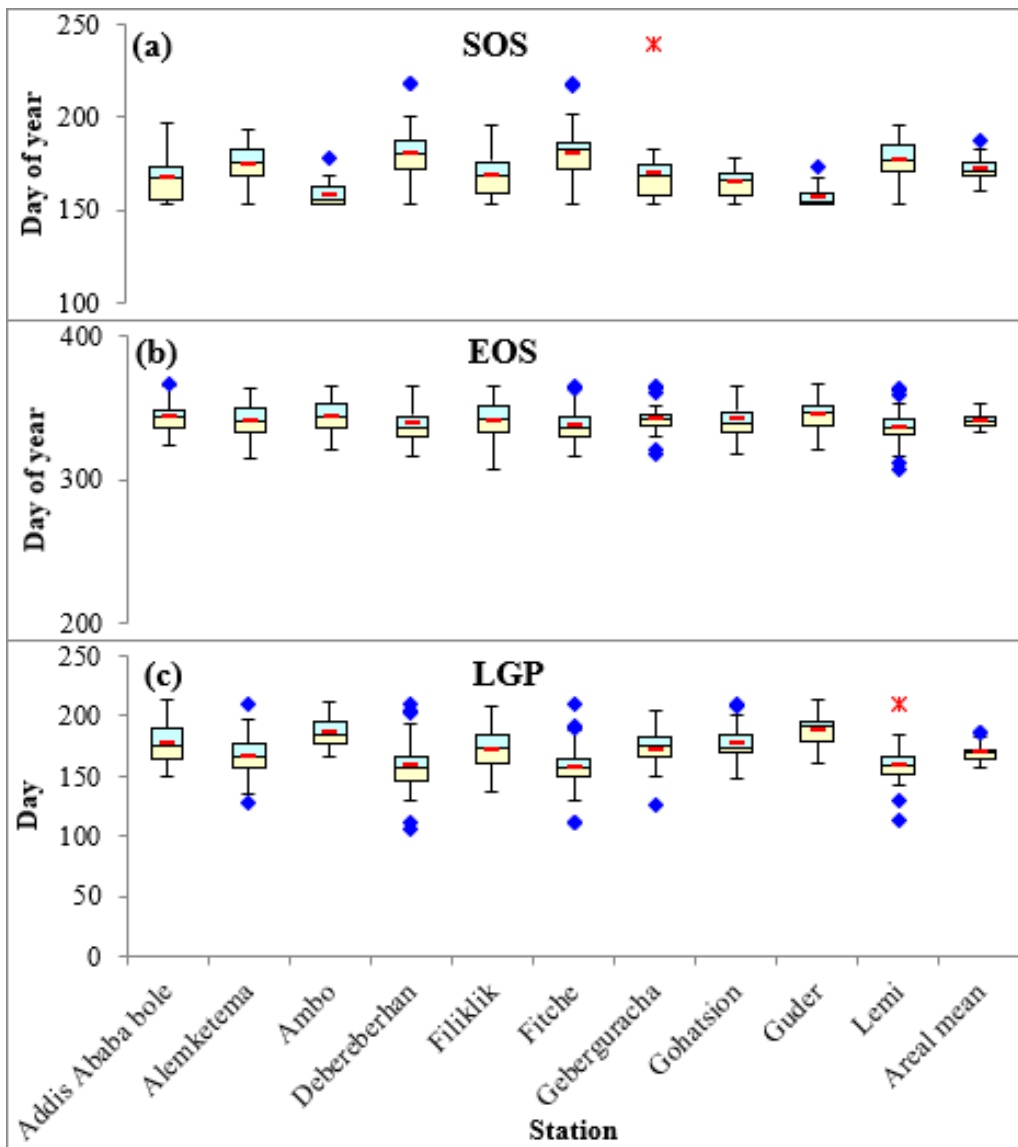
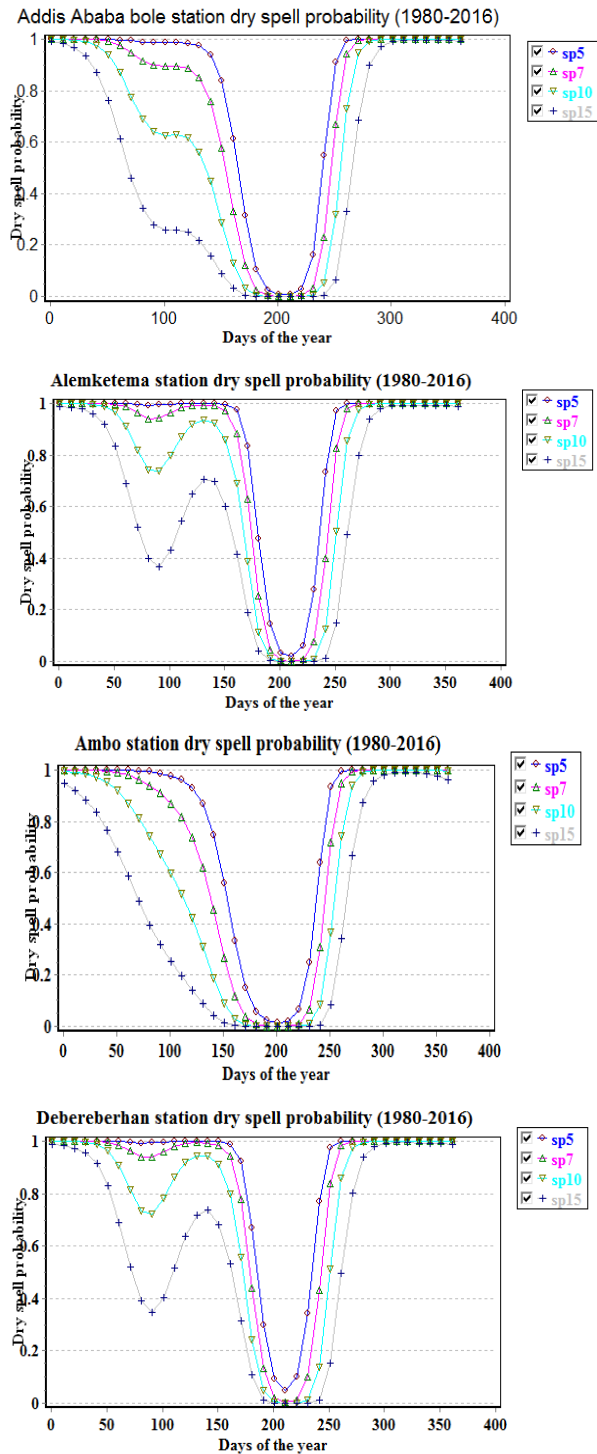
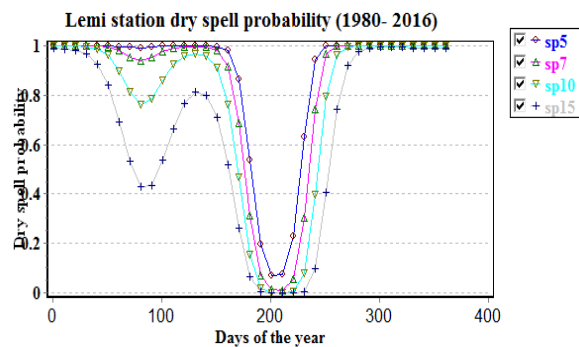
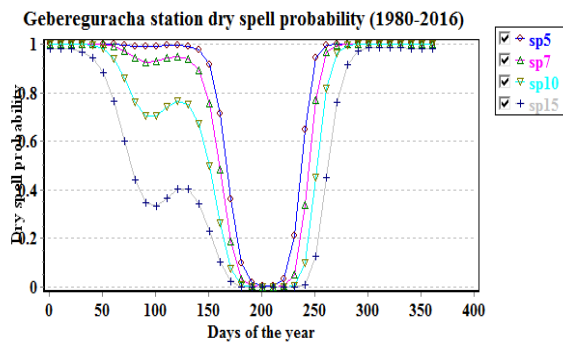
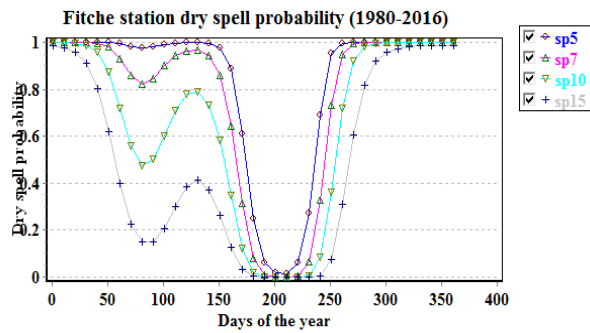
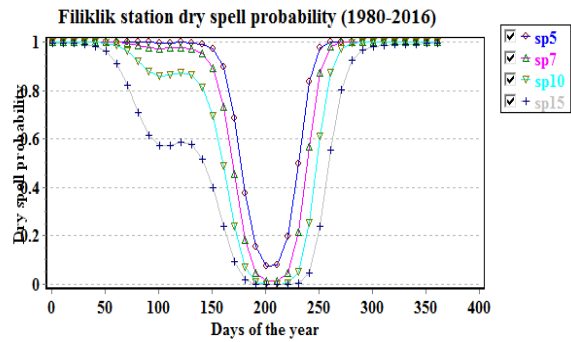


Figure 4. Summary statistics for rainfall onset (a), cessation (b) and length of growing period (c) for the *kiremt* season at eleven stations in central Ethiopia using rainfall records for the period 1980-2016.





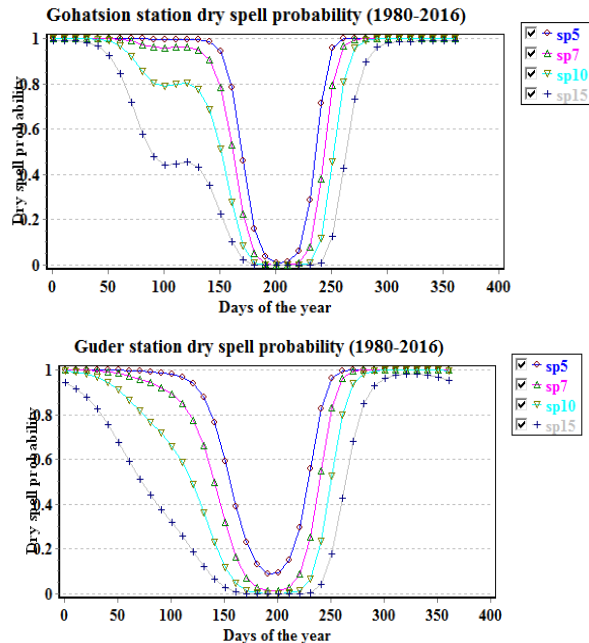
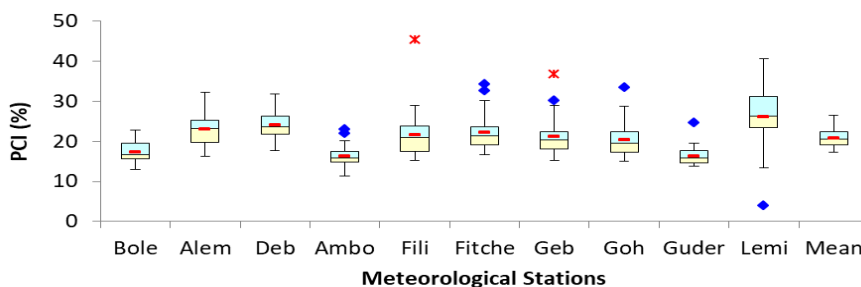


Figure 5. Probability of dry spells longer than 5, 7, 10 and 15 days at ten stations in Central Ethiopia for the period 1980-2016.

Rainfall concentration and anomaly indices

The calculated PCI showed a relatively better distribution of rainfall at Ambo, Guder, and Addis Ababa Bole stations across the months of the year (Fig. 6). PCI value at other stations, however, revealed that there was an indication of strong rainfall concentration in a few months of the year (Fig. 6). Fitch, Gebereguracha,

Gohatsion, and Filiklik stations were grouped under high to very high rainfall concentration, which indicated a poor annual distribution of the rainfall. High PCI values in high rainfall areas indicate more intense rainfall and runoff, soil erosion and waterlogging conditions which affect cereal crops production and food security in the central highlands of Ethiopia.



Key: Bole= Addis Ababa Bole; Deb= Debreberhan; Fili= Filiklik; Geb = Gebereguracha; Goh= Gohatsion, Alem = Alemketema.

Figure 6. Precipitation Concentration Index for ten stations in Central Ethiopia.

Based on the SAI values (Table 3) individual years at each site were grouped into extreme drought ($SAI < -1.65$), severe drought ($-1.28 > SAI > -1.65$), moderate drought ($-0.84 > SAI > -1.28$) and no drought ($SAI > -0.84$). Years in the early and mid-1980s were characterized by extreme drought at Fitcha, Debre Berehan and Ambo and were moderate drought at Gohatsion and Guder (Table 3). The year (annual) 2009 was an extreme drought at Gohatsion and Filiklik stations while it was a severe drought at Guder. The years 2002, 2003, 2012, 2014, 2015 and 2016 were either severe drought or moderate droughts depending on station considered (Table 30). In general, extreme drought were more frequent at Fitcha and Debre Berhan while severe droughts were common in Addis Ababa Bole. Compared to other

stations, the frequency of moderate drought years was higher at Addis Ababa Bole, Gohatsion, Guder, Lemi and Filiklik stations (Table 3). The SAI values also showed that 19, 32 and 30% of the years were categorized under extreme, severe and moderate drought classes, respectively. Out of the 37 years considered for this study, 38% of the years were non-drought years while 62% (2/3) of the years caused some level of drought in the region. Although the results clearly indicated large variation in the distribution of drought events across the study sites, it was clear that almost all stations experience moderate events at least two times in the last 37 years. Similar to this study, a spatial variation in the distribution of SAI was reported for nine stations in Southern Ethiopia (Wagesho, 2016).

Table 3. Standardized rainfall anomaly index classes for selected stations in central highlands of Ethiopia

Station	Extreme drought	Severe drought	Moderate drought	No drought
Addis Ababa Bole	-	1994, 2014, 2015	1987, 1997, 2002, 2004	All the rest years
Alemketema	1990	1991	2015	All the rest years
Fitcha	1980, 1983, 1985	1984	-	All the rest years
Debreberhan	1981, 1984, 1985	1987, 1989	2015	All the rest years
Gberguracha	1983, 1998	-	1991	All the rest years
Gohatsion	2002, 2009	1991	1981, 1984, 1992, 2004	All the rest years
Guder	-	2009	1980, 1984, 2015	All the rest years
Lemi	2011	1982, 2012	1980, 2002, 2015	All the rest years
Filiklik	2009	2003, 2016	1980, 2002, 2005	All the rest years
Ambo	1983, 1985	1982, 2015	-	All the rest years

Trend of extreme rainfall events

The results of the extreme rainfall event analysis are summarized in

Table 4. The results indicated that most of the indices did not show any significant trend at most of the stations. However, some extreme events indicated significant increasing

or decreasing trend at some sites. For example, Maximum 1-day rainfall (Rx1DAY) showed significant ($P<0.05$) increasing trend at Fitcha, Gebereguracha, and Gohatsion while it had a significant decreasing trend at Alemketema station. Similarly, Maximum 5-day (Rx5DAY) had a significant ($P<0.05$) trend at Gebereguracha but it had a significant ($P<0.05$) negative trend at Alemketema and Gohatsion stations (Table 4). Previous studies reported an increasing trend of Rx1DAY (Esayas *et al.*, 2018) and Rx5DAY (Worku *et al.*, 2018) in some stations such as Fitcha and Mendida and Alemketema in the central and highlands. A significant decreasing trend of Rx1DAY was also reported at Alemketema station (Worku *et al.*, 2018).

Unlike previous reports (Mekasha *et al.*, 2014; Esayas *et al.*, 2018; Worku *et al.*, 2018), most of the rainfall indices studied (SDII, R10mm, R25mm, CDD, CWD and R95p) did not show significant trend almost at all the sites (Table 4). The simple daily rainfall intensity index (SDII) showed a significant ($P<0.05$) decreasing trend at Lemi, while extremely heavy rainfall days index (R25mm) showed a significant ($P<0.01$) increasing trend at Fitcha (Table 4). The consecutive dry days index (CDD) did not show any significant trend at any of the stations whereas the consecutive wet days index (CWD) showed a significant ($P<0.01$) increasing trend at Debre Berhan only. The very wet days

(R95p) and extremely wet days (R99p) indices showed significant ($P<0.05$) increasing trend at Gebereguracha. However, the R99p was significantly declining trend at Alemketema and Gohatsion stations (Table 4). Annual total rainfall (PRCPTOT) did not show any significant trend at all the stations except for Fitcha and Gebereguracha, where it showed a significant ($P<0.05$) increasing trend. Alemketema, Fitcha, Gebereguracha, and Gohatsion were the stations where at least three rainfall extreme indices had a significant ($P<0.05$) increasing or decreasing trend. Out of the ten extreme event indices considered in this study, five of the indices showed a significant increasing trend at Gebereguracha followed by three increasing and three decreasing trends at Fitcha and Alemketema, respectively (Table 4).

Although the variation in the magnitude and trends of the extreme event makes it difficult to compare the results with previous studies, some studies support the current findings. For example, the increasing trend in rainfall amount at Fitcha was in line with two recent findings (see Degefu & Bewket, 2015; Esayas *et al.*, 2018). A study in north east Ethiopia also showed non-significant trend of most of the extreme events with some indices (e.g., consecutive wet days) significant only at a few stations (Mohammed *et al.*, 2018). Similarly, Esayas *et al.* (2018) reported non-significant trends for CDD and CWD indices in Southern Ethiopia. Shuai *et al.* (2018) reported a significant

decreasing trend of PRCPTOT in the western part of Oromia Regional State

while there was an increasing trend in the southern part of the Region.

Table 4. Trends in extremes rainfall events in selected stations in the Central Highlands of Ethiopia

Index [§]	Alem.	Bole	Ambo	Deb.	Fili.	Fitche	Geb.	Goha.	Guder	Lemi
Rx1DAY	-0.91*	-0.24	-0.17	0.33	-0.14	0.42*	0.63*	0.85**	0.02	0.61
Rx5DAY	-1.21*	-0.30	-0.12	0.13	0.44	0.15	1.18*	-1.21*	0.45	0.61
SDII	-0.06	-0.02	-0.02	0.00	0.04	0.03	0.03	-0.06	0.04	-0.13*
R10mm	-0.21	-0.06	0.09	0.07	0.83	0.17	0.27	-0.21	-0.10	0.45
R20mm	-0.05	-0.05	0.05	0.09	-0.14	0.13**	0.12	-0.05	0.18	-0.04
CDD	-0.12	1.08	-0.26	0.11	0.62	0.28	-0.39	-0.12	0.41	-1.78
CWD	-0.04	-0.05	0.39	0.31**	0.11	0.18	0.07	-0.04	-0.26	0.63
R95p	-3.51	-2.07	0.74	1.46	-4.35	1.83	5.46*	-3.51	2.64	1.99
R99p	-3.65*	-1.25	-0.82	0.43	-0.63	1.08	3.14**	-3.6**	-0.35	0.49
PRCPTOT	-4.54	-3.57	0.68	3.96	14.05	5.48**	8.39*	-4.54	0.69	7.83

Key: **, * Significant at 0.01 and 0.05 level, respectively. Alem= Alemketema, Bole= Addis Ababa bole; Deb= Debreberhan; Fili= Filiklik; Geb= Gebereguracha, Goha = Gohatsion. [§]Rx1DAY = maximum one day rainfall; Rx5DAY = maximum five days rainfall; SDII = simple daily intensity index; R10mm = Heavy rainfall days; R20mm = extremely heavy rainfall days; R95p = very wet days; R99p = extremely wet days; CDD = consecutive dry days; CWD = consecutive wet days; PRCPTOT = annual total rainfall.

Variation in cereal grains yield in Central Ethiopia

The simulated grain yield of wheat cultivars over the period 1984 to 2014 ranged from 1938 kg ha⁻¹ (cultivar Picaflor) to 6911 kg ha⁻¹ (cultivar Hidase) while that of Barley ranged from 3740 kg ha⁻¹ (cultivar Cros41/98) to 6003 kg ha⁻¹ (cultivar Shegee). The simulated wheat and barley yields were more variable at Debre Berhan

compared to those at Fitche, Ambo and Holeta, and wheat yields were more variable than that of barely at all stations (Table 5). A study in the central highlands of Ethiopian on wheat and barley indicated that rainfall variability at the different stages of the production period had a strong effect on yield of the two crops (Shumetie et al., 2017).

Table 5. Simulated mean grain yield (kg ha⁻¹) and coefficient of variation (CV) of wheat and barley at four stations in Central Ethiopia

Cultivar	Meteorological station							
	Debereberhan		Fitcha		Ambo		Holeta	
	Wheat							
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Alidoro	5231	29	6884	17	4451	10	5622	12
Hidase	5196	30	6911	16	4784	11	6038	10
Picaflor	2285	29	1938	22	3094	11	3524	14
Cros41/98 HB1307 Shegee	Barley							
	4453	27	5537	11	3740	7	3911	9
	4015	29	4521	11	4015	8	4290	10
	4693	31	6003	11	3994	12	4920	10

Source: Simulated yield by the authors for the period of (1984 to 2014).

Relations between climate variables and cereal yield

The grain yield of wheat and barley show a strong association with the seasonal rainfall amount (Table 6). Except for Fitcha, the yield of both wheat and barley had a significant ($P < 0.05$) positive correlation at Debre Berhan and a significant negative correlation at ($P < 0.01$) at Holeta and Ambo. The yield of the different wheat and barley cultivars also showed a significant ($P < 0.05$) correlation with mean maximum and

mean minimum temperatures although this varied among sites and the wheat and barley cultivars used for the study (Table 6). For example, the yield of most of the wheat and barley cultivar had a strong correlation with mean minimum temperature at Ambo, followed by Fitcha and Debre Berhan. On the other hand, yields of some wheat and barley cultivars were negatively and significantly correlated with mean maximum temperature at Debre Berhan and Ambo.

Table 6. Bivariate correlation coefficients between yields of wheat and barley cultivars and climatic variables at four stations in central Ethiopia

Station	Crop	Cultivar	Rainfall	Tmax	Tmin
Debereberhan	Wheat	Alidoro	0.45*	-0.38*	-0.35
		Picaflor	0.44*	-0.26	-0.25
		Hidase	0.42*	-0.28	-0.40*
	Barley	Cros41/98	0.36	-0.48**	-0.33
		HB1307	0.38*	-0.51**	-0.22
		Shegee	0.40*	-0.27	-0.38*
Fitcha	Wheat	Alidoro	-0.30	0.14	-0.40*
		Picaflor	-0.02	0.08	-0.06
		Hidase	-0.30	0.16	-0.37*
	Barley	Cros41/98	-0.30	0.03	-0.26
		HB1307	-0.33	0.14	-0.31
		Shegee	-0.41*	0.06	-0.34
Ambo	Wheat	Alidoro	-0.64**	-0.33	-0.49**
		Picaflor	-0.31	-0.31	-0.33
		Hidase	-0.57**	-0.45*	-0.96*
	Barley	Cros41/98	-0.71**	-0.14	-0.62**
		HB1307	-0.62**	-0.30	-0.52**
		Shegee	-0.49**	-0.49**	-0.30
Holeta	Wheat	Alidoro	-0.62**	-0.31	0.11
		Picaflor	-0.01	0.20	0.69**
		Hidase	-0.60**	-0.30	0.12
	Barley	Cros41/98	-0.66**	-0.28	-0.01
		HB1307	-0.70**	-0.06	0.22
		Shegee	-0.60**	-0.31	0.06

*, ** Correlation is significant at $P < 0.05$ and, $P < 0.01$ level; Tmax = Mean maximum temperature; Tmin = Mean minimum temperature.

The current results indicate that some of the wheat and barley cultivars grown in some of the study areas are affected by high maximum and minimum temperatures suggesting the need for carefully selecting crop varieties that are tolerant to high temperature conditions which will be more prevalent under climate change. Previous studies have shown a negative impact of climate change on wheat and barley crops mainly due to changing rainfall patterns and increasing temperatures (Evangelista et al., 2013). On average, seasonal rainfall amount explained 44, 21, 51 and 41% of the wheat yield and 38, 0.35, 61, 65% of the barley yield

variability at Debre Berhan, Fitcha, Ambo and Holeta, respectively. These effects are due to high variability of rainfall at Debre Berhan but due to waterlogging conditions at Ambo and Holeta. Wheat and barley are mostly grown on heavy clay soils in Ambo and Holeta areas and the heavy rainfall in July and August causing waterlogging conditions which reduce the yield of the crops. In those areas, practices such as shallow furrows and broad bed maker (BBM) technologies that help drain the water out of crop fields are needed to increase the productivity of the crops (Gebre and Le, 1996).

Conclusion

This study presented an analyses of climate variability and associated risks in relation to cereal crop production in central Ethiopia using historical rainfall records and simulated yield of wheat and barley. The rainfall variability parameters studied showed variations among the stations studied. The PCI results for most stations were lower for *kiremt* than for *belg* rainfall. The SAI values for most stations during the 1980-85, 1990, 1998, 2002, 2009 and 2011 years revealed occurrence of severe drought at most of the stations. Seasonal rainfall amount had a strong positive correlation with the yield of wheat and barley at Debre Berhan while it had a significant negative correlation at Holeta and Ambo. This indicates the need for designing and implementing location-specific crop and soil management practices to reduce yield losses either due to deficit or excess rainfall conditions. Negative correlation of some wheat and barley cultivar yields with mean maximum and minimum temperatures at some of the sites studied showed the challenges of climate variability in the productivity of wheat and barley in the near future. The study, in general, revealed that understanding the relationship between crop production and climate parameters is an important step towards identifying major productivity bottle necks and designing management and technological innovations to address

current and future climate related challenges at a local level.

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Calibration and Validation of CERES Model Sensitivity to Assess Impacts of Climate Change on Wheat Production

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Abstract

Crops exhibit specific responses in their growth and development to changes in local environment; including, soil climate and water. Since recently, crop simulation models have been widely put to use in assessing the impacts of a changing climate variables (temperature, rainfall, solar radiation, etc.) on crop production, while also targeting appropriate adaptation and mitigation responses. The objective of this study was to evaluate the Cropping System Model (CSM)-CERES-Wheat for its sensitivity to simulate growth and development responses of three wheat cultivars representing different maturity categories. Included cultivars were Hollandi, Meda Walabu and Sofumer which are widely cultivated in major wheat belts in Ethiopia. The CSM-CERES-Wheat simulation model was calibrated and validated against experimental data. The performance evaluation revealed that the model was able to simulate crop phenology (days to anthesis and maturity) and grain yield reasonably, with root mean square error ranging between 1.3 to 3.8 and 2.4 to 5.5 days for anthesis and maturity, respectively and, 5.2 and 6.5 qtha⁻¹ for grain yield. The index of agreement (d-statistics) between observed and simulated results were also in the higher range (>0.87). Overall, the resulting information from the study can be used for the impact studies and also for extrapolation of the resulting information into other similar agro-ecologies with less extra efforts; thus rendering the support to the crop-climate simulation modeling in reducing costs associated with multiple field experimentation and in optimizing the balance between the field trials and modeling studies, aiming at technology generation and promotional activities.

Introduction

The Intergovernmental Panel on Climate Change (IPCC) projected an average rise in a global surface temperature to the tune of 2.8°C through the 21st century, with best-guess ranging from 1.8 and 4.0°C (IPCC, 2012.). Global warming would alter natural climate and environmental systems in many ways,

leading to an increased frequency of extreme weather events, rising sea levels, a reversal of ocean currents, and changes in precipitation patterns.

The projected change in temperatures and rainfall patterns, as well as the associated impacts on water availability, pests and disease outbreak are, all likely to affect substantially the potential of agricultural production.

However, agricultural systems are complex, and understanding this complexity cannot be fully addressed with simple field experimentation alone, in which a very limited number of variables can be investigated under site specific conditions. Since recently, crop simulation models have been widely put to use in assessing the impacts of such risks through inputting key climate parameters into computer programs, while also scoping to search for an appropriate and effective means to develop climate risk solutions in a particular soil-climate zones (Challinor et al. 2009; Cooper et al. 2008; Mourice et al. 2014; Popova and Kercheva 2005; Tilman et al. 2011; Verdin et al. 2005).

Wheat is the most important food security crop in Ethiopia and it is grown on approximately 1.61 million hectares, with average production of 3.9 Mtyr^{-1} (CSA, 2014). Ethiopia is the second largest producer of wheat in Sub-Saharan Africa, following South Africa, while the crop is the second most consumed cereal in Ethiopia. Wheat crop accounts for approximately 11 percent of the national calorie intake ($200 \text{ kcal day}^{-1}$ in urban areas and $310 \text{ kcal day}^{-1}$ in rural areas). Wheat has versatile benefits in making various human foods, such as bread, biscuits, cakes, sandwich, etc. Additionally, wheat straw is commonly used as a roof thatching material and as animal feed (GAIN 2014). The yield gap of over 3 t ha^{-1} suggest the potential for increasing

production through improved land and crop management practices.

A range of responsive technologies have been identified in recent years, demonstrating the potential to increase resource use efficiency, reducing adverse environmental impacts and increasing crop productivity. We hypothesize that site specific evaluation and adaptation of these technologies can be assisted through the application of crop simulation modeling research. However, before deploying them for any impact assessment studies, the performance of the modeling tool should be tested for their sensitivity to capture local soil and climatic heterogeneity against the observed crop phenology and grain yield. The objective of this study was therefore to calibrate CERES-Wheat model for its sensitivity to capture growth and development process of wheat cultivars and to validate against the experimental data. Three wheat cultivars of different maturity categories namely; Hollandi, Meda walabu and Sofumer, which are widely cultivated in major wheat belts in Ethiopia, were used.

Data and Methodology

Description of CERES-Wheat model

The CERES-wheat (Estimation through Resource and Environment Synthesis) for wheat is available within the Decision Support System for Agrotechnology Transfer (DSSAT)

shell (Uehara and Tsuji 1993). The CERES-wheat module has the capability to simulate phenological development, biomass accumulation and partitioning, leaf area index, root to stem ratio, leaf growth and the water- and nitrogen balance from planting until harvest, grain yield on a daily time step, as influenced by weather, genetics, soil water, carbon, nitrogen, and planting, irrigation, and nitrogen fertilizer management (Timsina and Humphreys 2006a,b). Therefore, DSSAT/CERES-wheat module allows for the exploration of wider window of responses towards increasing yield, water and nitrogen use efficiency in wheat farming.

The minimum input data set that we used to simulate crop growth were cultivar specific parameters (genetic coefficients), soil properties, daily weather variables, crop growth and development data and agronomic management decisions; including planting date, planting density and depth.

Climate data

Daily maximum and minimum air temperature and rainfall data on record from the weather stations lying in close proximity to the experimental period were obtained from the

Ethiopian Institute of Agricultural Research (EIAR). Radiation data was obtained from the AgMIP (Agricultural Model Inter-comparison Project) climate forcing dataset based on the NASA Modern-Era Retrospective Analysis for Research and Applications (MERRA). The AgMERRA corrects climate data to gridded temperature and precipitation, incorporates satellite precipitation, and replaces solar radiation with NASA/GEWEX SRB to cover the base period 1980-2010 (Ruane et al. 2015).

Soil

Physical and chemical characteristics of soils (Table 1) were extracted from the International Soil Reference and Information Centre (ISRIC, 2013). Basic soil features required as input by the model were: silt content (%) and clay (%), soil organic carbon concentration (%), pH in water, lower limit of plant extractable soil water ($\text{cm}^3 \text{ cm}^{-3}$), drained upper limit ($\text{cm}^3 \text{ cm}^{-3}$), saturated upper limit ($\text{cm}^3 \text{ cm}^{-3}$), bulk density (g cm^{-3}), organic carbon (OC) for each soil layer. Other soil specific features such as color, run off value, slope and a fertility factor required as input by the model were determined.

Table 1 Physical and chemical characteristics of soils for the experiments

Soil depth (cm)	LL*	DUL*	SAT*	BD* (g/cm ³)	OC* (%)	Silt (%)	Clay (%)	pH
2	0.3	0.39	0.503	1.32	3.4	40	30	6.3
10	0.31	0.4	0.504	1.31	3.2	41	30	6.5
22	0.31	0.4	0.502	1.32	2.1	42	29	6.6
45	0.31	0.4	0.501	1.32	1.6	45	29	6.8
80	0.31	0.4	0.498	1.33	1.1	45	29	7.1
*150	0.3	0.39	0.49	1.34	0.8	44	29	7.4

Source : (ISRIC, 2013)

* LL; Lower limit, DUL; Drainage upper limit, SAT; Saturation upper limit, BD; bulk density; OC; Organic carbon

Management practices

The experimental data related to wheat production management practices for the period 2006-2011 were obtained from Sinana Agricultural Research Centre (SARC) of the Oromia Agricultural Research Institute (OARI). The experimental data were from fertilizer trials with rates ranging from zero (no fertilizer) to the recommended rates (100 kg) for the three wheat cultivars. Descriptions of the general cultivar specific properties

are presented in Table 2. The field trial was arranged in a 5×5 triple lattice design. The treatments with the fertilizer rates, i.e., 50 kg ha⁻¹ urea and 100 kg ha⁻¹ diammonium phosphate of total 41 kg N ha⁻¹ and 46 kg P ha⁻¹ were used. Initial conditions, such as previous crop, planting depth and planting dates, row spacing and plant population were collected from published sources and then set as input in the experimental design of simulations.

Table 2 Description of general cultivar specific properties

Variety name	Yield (Qtha ⁻¹)	Days to		Plant height (cm)	Annual rainfall (mm)	Year of release
		Anthesis	Maturity			
Meda walabu	29-43	76	136	95	400-950	2000
Sofumar	23-42	73	134	97	400-950	2000
Hollandi (Local)	15-18	78	135	96	750-1000	

Source: Sinana Agricultural Research Centre (SARC)

Model calibration

Model calibration is an adjustment of crop growth parameters, so that simulated values accurately capture the observed data. The genetic coefficients' that influence the

occurrence of developmental stages in the DSSAT was derived iteratively by manipulating the relevant coefficients to achieve good overall agreement between simulated and observed values of phenological and growth

events. To calibrate the cultivar coefficients (Hunt *et al.* 1993), the Generalized GENCALC software package, which is one of the two genetic coefficient estimator tools in DSSAT was used. The CSM– CERES-Wheat model has 7 cultivar specific coefficients that define growth and development characteristics of wheat cultivar (Table 3). Four of the coefficients define the life cycle and

development characteristics, while the other three defining the growth characteristics (Jones *et al.* 2003). The DSSAT model was calibrated using three growing season data (2007, 2009 and 2011) for Meda walabu and Hollandi cultivars; while data from 2007, 2009 and 2010 were utilized for Sofumer cultivar.

Table 3 CERES-Wheat genetic coefficients

Abbreviation	Definition
P1V	Vernalization sensitivity coefficient (%/ of unfulfilled vernalization)
P1D	Photoperiod sensitivity coefficient (% reduction/h near threshold)
P5	Thermal time from the onset of linear fill to maturity (°C d)
PHINT	Thermal time between the appearance of leaf tips (°C d)
G1	Kernel number per unit stem + spike weight at anthesis (#/g)
G2	Potential kernel growth rate (mg/(kernel d))
G3	Tiller death coefficient. Standard stem + spike weight when elongation ceases (g)

Source (Jones *et al.*, 2003b)

In the search for genetic coefficient, GENCALC starts with the initial “default” values for each cultivar coefficient. The value for a given cultivar coefficient varies, relative to one or more simulated and observed crop measurements. The algorithm searches the crop model output file and, based on the difference between simulated and actual target variables. Up on finding a good fit to each observation, the GENCALC averages the coefficients and calculates the root mean square error (RMSE) (Wallach and Goffinet 1987) across all the trials included. GENCALC uses an interactive procedure, in which the user changes the cultivar coefficient

step to minimize the error. The search was ended upon achieving the parameters providing the lowest RMSE for a single target trait or the lowest average normalized root mean square error (Loague and Green 1991) for multiple target traits.

The accuracy of the procedure used to estimate the cultivar coefficients was determined by comparing the simulated average values with the corresponding observed mean values for the days to anthesis and harvest maturity as well as final grain yields, and by the values of the RMSE. A low value of RMSE indicates goodness of fit between the simulated and observed

values. The RMSE was computed using the following equation:

$$RMSE = \left(\sum_{i=1}^n (Y_i - X_i)^2 / n \right)^{1/2}$$

where, n : number of observations, Y_i : predicted value, X_i : observed value for the i^{th} measurement

Validation of the cultivar coefficients

After calibration, the model sensitivity was evaluated using an independent data sets collected over three years (2006, 2008 and 2010) for Meda Walabu and Hollandi cultivars, and two years (2006 and 2008) for Sofumer cultivar. The variables tested include the key crop phenological dates (anthesis and harvest maturity)

and the yield components, as recommended by Hunt *et al.* (1993). Statistical indicators, i.e. the root mean square error (RMSE), index of agreement (d) (Willmott *et al.* 1985) and coefficient of determination (R^2) were used for evaluating the goodness of fit between the observed and simulated values. Low values for RMSE and d-values, were employed to detect goodness of fit.

$$d = 1 - \left[\frac{\sum_{i=1}^n (Y_i - X_i)^2}{\sum_{i=1}^n (|Y_i - \bar{X}| + |X_i - \bar{X}|)^2} \right], \quad 0 \leq d \leq 1$$

where, n : number of observations, Y_i : predicted value, X_i : observed value for the i^{th} measurement, $\bar{X}$ the overall mean of observed values.

Due to insufficient experimental data, R^2 were not calculated for Sofumer cultivar.

Results

Table 4 below shows the values of the seven DSSAT genetic coefficients for the three wheat cultivars by fitting the model outputs against the data from the established experimental trials. The result indicated that the cultivar coefficients that were derived from model calibration have simulated both

durations from planting to days to anthesis (dA) and from planting to maturity (dM) quite adequately; showing how the CERES-Wheat model was sensitive to capture the responses of the three wheat cultivars to the variable climate, soil, fertilizer and water.

Table 4 Calibrated genetic coefficients of wheat cultivars

Cultivar	P1V	P1D	P5	G1	G2	G3	PHINT
Meda Walabu	28.97	53.3	543.2	35.23	20.34	1	95
Sofumer	34.09	13.11	507	13.49	44.3	1	95
Hollandi	29.27	46.27	525.1	34.97	17.52	1	95

Comparisons among the observed and simulated values for the dA, dM and for the grain yield, based on the calibration data are highlighted using the 1:1 trend line. Further, the RMSE values for dA and dM were low (0.8 to 3.9 days), with the highest degrees of association ($R^2 > 0.96$) being established among observed and simulated data. The corresponding observed d-statistics also supports the

results for both traits, except for days to anthesis, in case of Sofumer ($d=0.48$). Similarly, the simulation of grain yield showed good agreement with the observed values, as could be explained by the RMSE values, ranging from 5.9 to 7.2 qtha^{-1} . Yet, the d values were relatively lower for the grain yield (mean $d=0.59$), compared to the other traits, in particular for Meda Walabu cultivar ($d=0.43$).

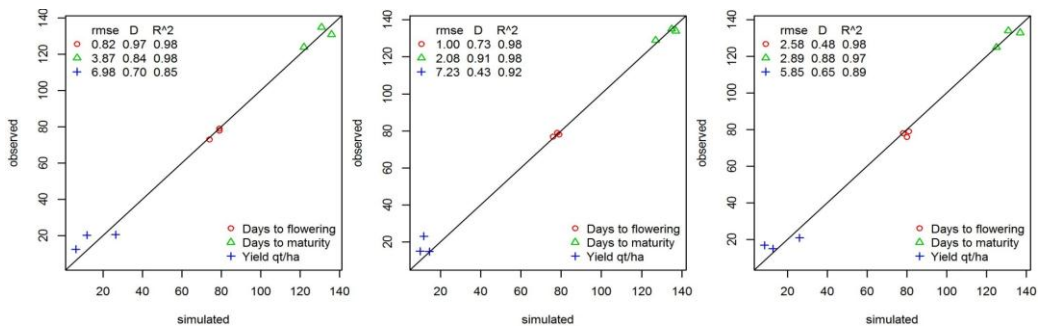


Figure 1. Comparison of simulated to measured data during model calibration for the three cultivars (from left to right, Hollandi, Meda walabu and Sofumer, respectively)

The validation test using independent data sets were in good agreement with the corresponding observed values for all experiments for dA and dM, with lowest mean RMSE of 2.8 and 3.6 days, respectively. The strong mean association ($R^2=0.83$ and $d=0.82$) for dM, as well as the moderately high mean value of ($R^2 = 0.66$ and $d=0.69$) for dA shows the validity of the

results. This goodness of fit statistic can be compared favorably to those reported by (Rezzoug *et al.* 2008) for the simulation of winter wheat with DSSAT, in which the model achieved RMSEs of 4.4 and 3.5 days for the anthesis and maturity dates, respectively. Finally, for the grain yield, the RMSE was ranged between 5.2 and 6.5 qtha^{-1} for validation. In

general, the model could simulate both crop development traits and final grain yields quite well for the wheat

varieties under study, the result of which can be used for selection of compatible adaptation responses.

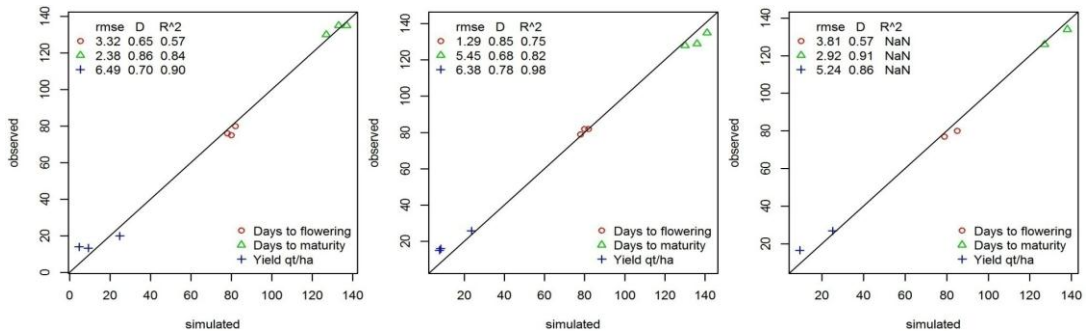


Figure 2. Comparison of simulated to measured data during model validation for the three cultivars (from left to right: Hollandi, Meda walabu and Sofumer, respectively)

Figure 3 also highlights little difference in model performance between the calibration and validation phases. The RMSE between simulated and observed anthesis dates ranging between 0.8 and 2.6 days in calibration and between 1.3 and 3.8 days for validation. The prediction of maturity also showed similar result, with RMSE ranging from 2.1 and 3.9 days for calibration and between 2.4 and 5.5

days in the validation phase. For the grain yield, the RMSE was ranged between 5.9 and 7.2 qha⁻¹ for model calibration and between 5.2 and 6.5 qha⁻¹ for validation. In general, the model could simulate both crop development and final grain yields quite well for the wheat varieties under study, the result of which can be used for selection of compatible adaptation responses.

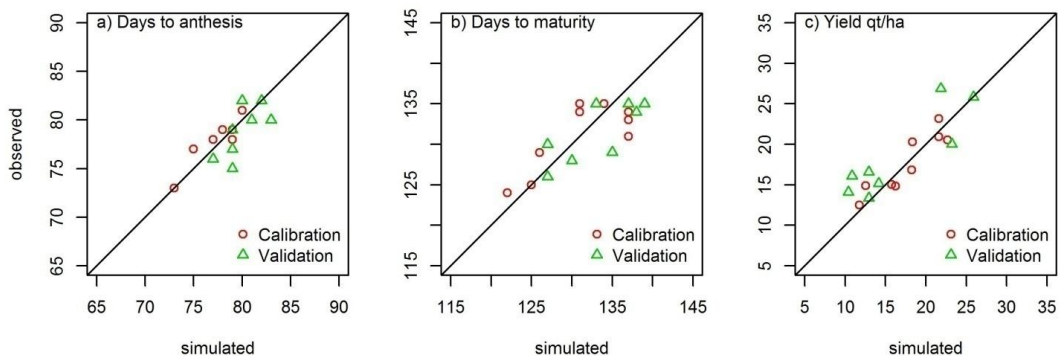


Figure 3 Comparison of simulated and observed data for a) anthesis, b) maturity, c) grain yield for the three cultivars for calibration (circle) and testing years (triangle).

Discussion

The iterative simulation run through trial-and-error adjustments in matching the observed phenology and yield with those simulated showed how the model performed quite well in simulating both crop development and final grain yields. For the days to anthesis, this can be best explained by the respective lower RMSE of 1.5 days for model calibration and the validation data of 2.8 days. For the days to crop maturity phase, similar lower RMSE of 2.9 days were obtained for the calibration and 3.6 days for validation. Timsina & Humphreys (2006a,b) also found comparable results, with DSSAT v3.5 predicted anthesis and maturity with a RMSE of 4.49 and 5.08 days, respectively.

Similarly, the model was able to simulate the final grain yield quite well, with mean RMSE of 6.7 and 6.0 qtha⁻¹ during model calibration and validation, respectively. For comparison, Jamieson et al. (1998) also studied 5 different wheat models in Australia, including CERES-Wheat, and found RMSE of 9 qtha⁻¹. Calibrating and validating 9 wheat cultivars in Algeria. Rezzoug et al. (2008) also reported similar results with DSSAT v4.0 predicted final yield with RMSE of 7.6 qha⁻¹ and R² of 0.71.

Our study also showed that there were little difference in model performance between the calibration and

independent validation phases. The RMSE between simulated and observed days to anthesis ranged between 0.8 and 2.6 days in the calibration and between 1.3 and 3.8 days for testing.

The prediction of days to maturity also showed similar result, with RMSE ranging from 2.1 and 3.9 days for the calibration and between 2.4 and 5.5 days in the testing phase. For the grain yield, the RMSE was ranged between 5.9 and 7.2 qtha⁻¹ for the calibration and between 5.2 and 6.5 qha⁻¹ for model validation. The rather low mean values of d for the grain yield could be attributed to the fact that the genetic coefficients were obtained from a limited field observations.

Conclusion and Recommendation

In this study, we assessed the capabilities of CERES-Wheat model for simulating wheat growth and yield at three major wheat producing areas of Ethiopia. Results showed that the model accurately reproduced independent empirical data both for the developmental and growth traits. However, to make the best of crop simulation modeling in practical decisions, lack of historically collected experimental crop data is a challenge, and research has to focus on collection of biomass and related parameters on locally adapted wheat cultivars.

Overall, the resulting information from the study can be used for the impact studies and also for extrapolation of results into other similar agroecologies with little extra efforts; thus rendering the support to the crop-climate simulation modeling in reducing costs associated with multiple field experimentation and in optimizing the balance between the field trials and modeling studies, aiming at technology generation and promotion efforts. The model can also be further improved for simulation of other parameters, including leaf size, effective tillers, leaf area and grain sizes and biomass for more certainty in predicting crop development and growth.

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Release and Registration of New Bread Wheat Variety “Galan” For Bale Highland and Similar Agro-Ecologies

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Abstract

The name “**Galan**” designated by pedigree **ETBW 8003** is given to the newly released bread wheat (*Triticum aestivum* L.) variety. The variety was released by Sinana Agricultural Research Center on June 2019. **Galan** was primarily introduced through International Center for Agricultural Research in the Dry Areas (ICARDA) for evaluation under disease screening nursery trials during 2013 at Sinana. This variety is a medium maturity & its mean grain yield performance ranged from 4.87 to 5.93 t ha⁻¹ on research field and 4.21 to 5.37 t ha⁻¹ on farmers field. Stability analyses indicated that **Galan** is relatively better stable than the other genotypes used in the study. It is moderately susceptible reaction to both stem rust and yellow rust with 10% and 15% severity, respectively. The result of quality analyses showed that gluten, protein and zeleny index of **Galan** is 32.9%, 14.4% and 72.5%, respectively, which is acceptable and fulfill standard requirement of food factories.

Keywords: *Galan*; Grain yield; Stable genotype; Yield performance

Introduction

Ethiopia is the leading wheat producer in Sub Saharan Africa and total production is 4.64 million tons (CSA, 2018). The national average of wheat productivity is estimated to be 2.74 t ha⁻¹ (CSA, 2018), which is below the world average of 3.0 t ha⁻¹ (Hawkesford *et al.*, 2013). Wheat produced in Ethiopia is used mainly for domestic food consumption, seed and raw material for agro-industries. It accounts for about 10-15% of all the calories consumed in the country (Berhane *et al.*, 2011). Moreover,

estimated total wheat consumption (for food, seed and industrial use) is rapidly increasing at the national level (CSA, 2018). According to GAIN (2013), wheat consumption growth is higher in urban areas than other area due to higher population growth, changes in life style, and the rising prices for *teff*. However, production and productivity of wheat is highly constrained by the limited availability of high yielding and disease resistant varieties, which hampered our attempt to bridge the gap for the huge demand at national level. Therefore, the objective of this study was to evaluate

and release stable high yielding and disease resistant/tolerant bread wheat variety for the highlands of Bale and other similar agro-ecology.

Variety origin and evaluation

Galan is ICARDA material and it was formerly introduced to Sinana Agricultural Research Center as bread wheat key location disease screening nursery 2013 (BWKLDN-13) from Kulumsa Agricultural Research Center during 2013/14 cropping season. This genotype showed good field performance and then promoted to bread wheat preliminary yield trial 2014 (BWPYT-14). It was evaluated together with 35 other genotypes including standard checks at Sinana, Agarfa and Goba during 2015 and 2016 main growing season. **Galan** (ETBW 8003) showed better performance for grain yield and major disease than all the tested genotypes and checks.

Morphological and Agronomical characters

Galan has erect juvenile plant growth, an erected flag leaf with white glumes. The spike is owned, mid-dense, and tapering. The kernel is amber color and ovate in shape with angular cheeks and a narrow, mid deep crease. The

brush on the kernel has a collar and is medium in length. **Galan** is relatively medium-tall variety with 102cm height but less than Sanate with erected type upright growth habit and low tillering capacity. This variety is a medium maturing cultivar; it takes an average of 68 days from planting to heading and 138 days to mature in Bale highland. The detail of morphological and agronomical character is shown in Table 1.

Grain Yield Stability Performance

Mean yield performance of variety **Galan** ranged from 4.87 to 5.93 t ha⁻¹ on research field and 4.21 to 5.37 t ha⁻¹ on farmers field. It has yield advantage of 22.7% over standard check Dambal, and 99.5% over Mada walabu. The analysis based on AMMI stability value revealed that **Galan** genotype is relatively more stable than other genotypes used in the study. Results from GGE biplot analysis also showed that **Galan** (genotype #28) fall close to the central circle away from vertical mean line, indicating its high yield potential, relative stability and wider adaptability compared to the other genotypes (Fig.1).

Table 1. Agronomic and morphological descriptors of the released bread wheat

Variety Name	Galan (ETBW 8003)
Adaptation area	Highlands of Bale and similar agro ecology
Altitude (m.a.s.l)	2200-2500
Rainfall (ml)	750-1500
Fertilizer (kg/ha)	
P ₂ O ₅	41
N	46
Seed rate (kg/ha)	150
Days to heading	68
Days to mature	138
1000 seed weight(g)	49.2
Hectoliter weight(kg/hl)	84.3
Plant height(cm):	102
Yield (t ha ⁻¹)	
Research field	4.87-5.93
Farmers' field	4.21-5.37
Seed color	Amber
Growth habit	Erect
Spike density	Medium density
Seed shape	Oval shape

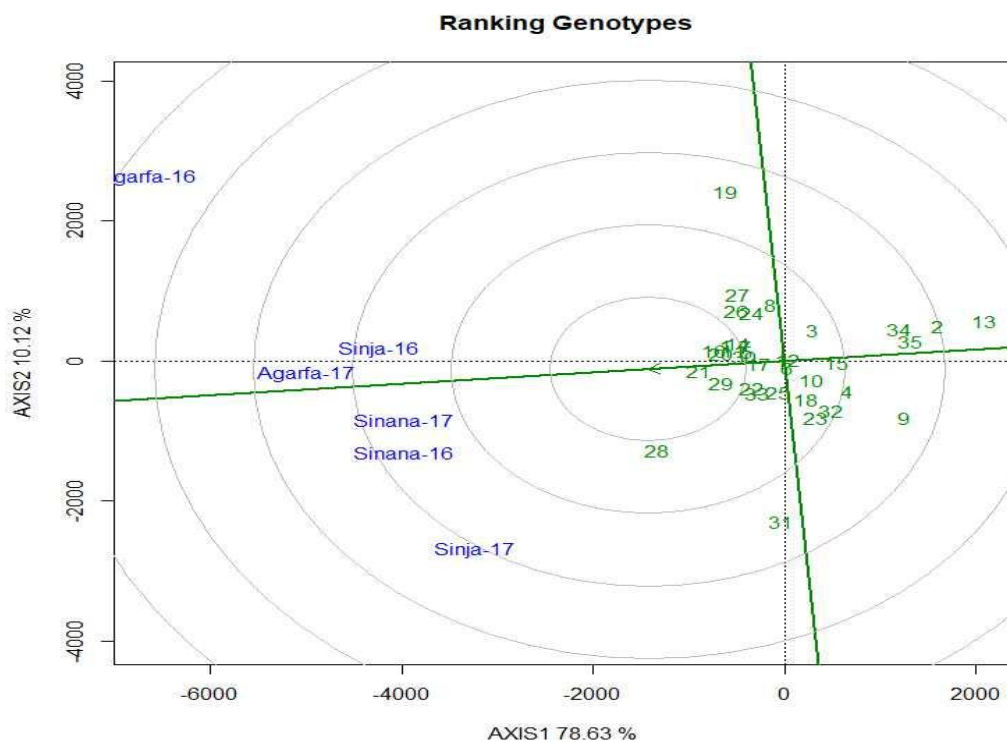


Figure 1: Ranking ideal genotypes for ideal environment

Disease Reaction

The variety **Galan** has moderately susceptible reaction to both stem rust and yellow rust with 10% and 15% severity, respectively (Table 2).

Whereas, the maximum score of stem rust overall location for the checks Dambal, Mada walabu and Holandi were 5ms, 10ms and 40s; but yellow rust was scored 30s, 40s and 40s, respectively.

Table 2. The maximum Disease Reaction of variety **Galan** and checks

SN	Variety	Sr	Yr	Lr
1	Dambal (standard check)	5ms	30s	0
2	Galan (ETBW 8003)	10ms	15ms	0
3	Mada walabu (standard check)	10ms	40s	0
4	Holandi (local check)	40s	40s	0

Key: Sr= stem rust (%), Yr= Yellow rust (%), Lr= leaf rust (%), s= susceptible reaction, ms= moderately susceptible reaction

Quality parameters

The nutritional quality analyses are presented in table 3. The result showed that variety **Galan** had high thousand kernel and hectoliter weight. **Galan**

showed best quality for gluten content, protein content and zeleny index than the standard checks (Table 3). Therefore, this variety could be well accepted by food factories as its quality meet required standard.

Table 3. Bread wheat physical and chemical quality Analysis

Variety	TKW (gm)	HLW (hl/kg)	Gluten (%)	Moisture (%)	Protein (%)	Zeleny index (%)
Dambal	41.2	81.7	30.5	12.1	13.3	65.0
Galan (released variety)	49.2	84.3	32.9	12.1	14.4	72.5
Mada walabu (standard check)	35.8	77.2	18.8	12.0	10.1	47.4
Holandi (local check)	36.4	77.5	-	-	-	-

Key: TKW= thousand kernel weight, HLW= hectoliter weight

Availability/variety maintenance

Sinana Agricultural Research Center will be maintaining breeder seed of **Galan**. Basic and Pre basic seed will be multiplied by SARC and other private or public seed producing enterprises. Seed sample will be deposited in the Ethiopian Biodiversity

Institute (EBI) for genetic resources preservation.

Conclusion and Recommendation

Galan is fully released for the highlands of Bale and similar agro-ecologies because it has high grain yield and quality, tolerant to disease, stable yield performance across

environments and good agronomic trait. Therefore, smallholder farmers, private and public seed enterprises and other wheat producers in Bale highland and similar agro ecology can produce ***Galan*** with its full management recommendation.

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The Release and Registration of “Yale” (Ew002 X Dicho 5-3) Sesame (*Sesamum indicum* L.) Variety

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Abstract

Yale is a name given to a newly released sesame variety developed by Bako Agricultural Research Center and released in June 2019. Selection of this variety was made among recombinant inbred lines of sesame developed through pedigree breeding method. The two parents of the selected variety were collected from western Ethiopia. It was selected out of thirteen lines that were tested along with two standard checks viz., ‘Chalasa’ and ‘Walin’ at seven locations in year 2017 and at five locations in year 2018. Yale was the best high yielding variety with mean grain yield of 851 kg ha⁻¹. This variety was the most stable among all lines for its grain yield performance and has resistance to bacterial blight, high uniformity with maximum oil content (55.2%). It was identified to be the farmers’ preferred variety.

Key words: Grain yield stability, Oil content, Sesame, Variety development

Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop grown for local consumption and export in Ethiopia, and ranks first in area of production and as export crop among oilseed crops grown in the country (CSA, 2015). Ethiopia is among the world’s top five producers of sesame and the third largest world exporter of the crop (Wijnands *et al.*, 2011). Sesame production is increasing from year to year, which is mainly driven by increasingly high export

market demand and availability of suitable agro-ecologies (Zerihun Jaleta, 2012).

Despite the potential for increasing the production and productivity of the crop, there are a number of challenges inhibiting sesame production and productivity. Among the many production constraints, the most important include limited availability of improved and stable high yielding varieties with desirable agronomic qualities, diseases/pests resistant for different agro-ecologies, and poor seed supply system (Negash

Geleta, 2015). Western Ethiopia is one of the potential areas of the country where sesame is largely produced. Sesame breeding for this part of the country started in year 2005. So far, four varieties were released by simple selection method. As an effort of sesame breeding program for western Ethiopia, the new sesame variety, Yale was developed by crossing potential breeding lines.

Varietal Origin and Evaluation

The parents of this variety were collected from Western Ethiopia (east and west Wollega). Parent selection for crossing purpose was done in year 2010 and crossing of the parents was done in 2011 main season. Out of large number of crosses, thirteen recombinant inbred lines were developed by pedigree breeding method. The new variety, Yale was tested at multi location together with fifteen genotypes including two standard checks, 'Chalasa' and 'Walin' during 2017 & 2018 cropping season (Annex Table 2).

Agronomic and Morphological Characters

Detail of the agronomic and morphological characters of the new sesame variety was presented in Annex Table 1. This variety has determinate growth type. It has erect growth habit and the stem is green in

color with alternate stem branching habit. It has basal branching character and thus better lodging resistance than the standard checks. It has opposite leaf arrangement with oval leaf shape. It has dentate basal leaf margin. Its calyx tip is purple in color.

Yield Performance

The variety *Yala* gave grain yield ranging from 600 to 1192 kg ha⁻¹. The result showed that the variety was the best for its grain yield (851 kg ha⁻¹). It has 53.6 and 23.1% grain yield advantage over the standard check, *Chalasa* and *Walin*, respectively.

Stability Performance

Based on the AMMI Stability Value (ASV), the new variety, Yale ranked first for its stability for grain yield performance and the GGE biplot confirmed that the variety is the most stable (Chemedda Daba and Solomon Bekele, 2018).

Disease Reaction

Bacterial blight is the most yield-limiting factor for sesame production in western Ethiopia as the disease is favored by the effect of high humidity and rainfall condition in the area. In addition to higher yield, this variety showed better resistance to bacterial blight than the standard checks.

Quality Analysis

Oil content analysis of the varieties indicated that new variety, Yale was

the best with 55.2%, while the oil content of the standard checks were 54.7 and 54.7% for *Chalasa* and *Walini*, respectively.

Adaptation

The new variety, *Yale* is adapted to Angar, Gute, Bako, Boneya, Hagalo, Uke and Wama areas and similar agro-ecologies in altitude ranging from 1300 to 1650 meters above sea level. This variety can be grown in high rainfall areas where bacterial blight is a problem for sesame production.

Conclusions

Yale is the first variety that was developed by Bako Agricultural Research Center through the pedigree breeding method. It is stable variety across locations with high grain yield performance and high oil content. A new variety *Yale* was resistance to bacterial blight with good uniformity in height and resistant to lodging. *Yale* was released in June 2019 for western Oromia and similar agro-ecologies.

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Annex Table 1. Agronomic and morphological characters of the new sesame variety

1	Variety	Yale (EW002 x Dicho 5-3)
2	Agronomic & morphological characters	
	2.1.Adaptionarea:	Angar-Gute, Bako, Boneya, Hagalo, Uke,Wama& similar agro-ecologies
	Altitude(ma.s.l	1300-1650
	Rainfall (mm)	800-1100
	2.2.Fertilizerrate(kg/ha)	
	NPS	100
	Urea	50
	2.3.Seedrate(kg/ha)	5
	2.4.Spacing(cm)	40x5(InterandIntrarows)
	2.5.Plantingdate	Late May to early June
	2.6.Daystoflowering	56-66
	2.7.Daystomaturity	115-125
	2.8.Plantheight(cm)	116-140
	2.9.Branch height (cm)	15-40
	2.10.Capsule height (cm)	45- 70
	2.11.1000seedweight(g)	2-3
	2.12.Seedcolor(g)	White
	2.13.Oilcontent(%)	55.3
	2.14.Yield(Kg/ha)	
	Research field	600-1100
	Farmers field	700-900
3	Year of release	2019
4	Breeder seed maintainer	OARI/BARC

Annex Table 2. Mean grain yield (kg ha⁻¹) of 15 sesame genotypes across two years (2017 and 2018) and locations

No.	Variety	Year one (2017)								Year two (2018)				Mean
		Angar	Lugo	Uke	Wama	Bako	Boshe	Boneya	Bako	Hagalo	Lugo	Uke	Wama	
1	EW002 x Obsa 1-1	906	734	993	598	668	391	453	721	934	871	692	695	721
2	EW002 x Obsa16-1	729	908	643	776	505	414	494	900	786	467	567	469	638
3	EW002 x Obsa22-1	1042	778	957	801	682	644	666	514	570	870	770	932	769
4	Obsa x Dicho19-11	646	627	795	504	438	501	571	749	689	586	772	666	629
5	Obsa x Dicho19-3	1040	930	930	651	576	403	575	723	837	1013	809	937	785
6	OBSA x Dicho 27-1	839	600	738	519	358	469	600	901	689	360	683	376	594
7	EW002 x Dicho 1-1	569	720	558	647	444	311	538	690	246	453	611	473	522
8	EW002 x Dicho 5-3	867	1063	908	758	603	523	666	1192	960	943	935	799	851
9	EW002 x Dicho 12-1	614	743	466	712	316	327	512	360	579	450	542	400	502
10	EW002 x Dicho 17-2	619	640	436	706	278	628	481	968	724	566	430	638	593
11	EW002 x EW006 (3-1)	617	734	724	834	363	514	571	674	784	454	642	687	633
12	Dicho x EW006 (9-1)	450	809	678	576	444	374	313	610	677	586	649	391	546
13	Dicho x EW006 (9-1)	769	980	647	734	624	374	621	378	561	665	758	682	649
14	Chalasa(standard check)	527	732	503	620	390	501	446	709	634	575	575	435	554
15	Walini (standard check)	946	909	845	569	540	449	574	630	509	770	782	766	691
	Mean	745	794	721	667	482	455	539	715	679	642	681	623	

The Release and Registration of “Fadis-01” Sorghum [*Sorghum bicolor* (L.) *Monech*] Variety

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Abstract

Fadis-01 is a common name given for Sorghum [Sorghum bicolor (L.) Monech] variety with pedigree designation of M-36121 X P-9403. The variety was developed and released by Fadis agricultural research center for yield and Striga resistance/tolerance in the lowlands of Hararghe. It was verified at on farm and sub site of Fadis, Babile, Harari, and Dire Dawa areas in 2018 main cropping season and released for yield and Striga resistance on June 2019. The variety showed the highest mean yield (3.9 tons ha⁻¹) among all genotypes evaluated at multi location and 25% yield advantage over the standard check (Hormat), early maturing (120 days) and showed stable yield performance across locations and years. It has good level of Striga resistance, which is a major threat to sorghum production in the study areas and the vicinities.

Keywords: *Sorghum [Sorghum bicolor (L.)*, Striga resistance,

Introduction

Sorghum is among the main staple food crop in Ethiopia, ranking fourth after Tef, maize, and wheat, both in area coverage and production (CSA, 2016). It is adapted to a wide range of environment and hence can be produced in the highlands, medium altitude and lowland areas. The ability of the crop to withstand drought stress and reasonable yields under adverse environmental conditions made it important food security crop in arid and semi-arid lowlands. Its residues after harvest and green plants also

provide sources of animal feed, building materials and fuel particularly in the semi-arid tropics (ICRISAT, 1997).

Even though, the crop is important for the Eastern Hararghe zone particularly to Babile, Fedis, Harari and Dire Dawa, several factors constrained productivity such as limited availability of improved varieties, striga infestation, moisture stress and other edaphic factors. Developing high yielding variety tolerant to *Striga* infestation and the fluctuating weather condition is the major target of sorghum improvement program.

Therefore, the objective of this activity was to release high yielding & striga resistant/tolerant variety for sorghum growing lowland areas of Eastern Ethiopia.

Variety origin and evaluation

Fadis-01 (M-36121 X P-9403) was originally introduced from Melkassa sorghum improvement research program and developed through pure line selection method. Six selected genotypes were evaluated at multi locations against the standard check (Hormat) for three consecutive (2014, 2015 and 2016) main cropping seasons at Fadis and Babile (Erer) sub sites. It was verified on farmers' field and research sub sites at Fadis, Babile, Harari and Dire Dawa areas during 2018 main cropping season. The variety was evaluated and officially released on June 2019 for its stable high yield performance and *Striga* resistant for wider production in the lowlands of Eastern Hararghe and areas with similar agro-ecologies.

Varietal Characteristics

Fadis-01 has semi compact head type, average days to flowering (77 days) and maturity (120 days) (Table 1). The variety has medium plant height (160 cm). This character is preferred by the local community as the larger biomass is used for multipurpose (livestock feed, fire wood and construction). The

seed color is white and has average thousand-kernel weight of 30 g.

Yield Performance

The combined mean grain yield (3.9 tons ha⁻¹) of this variety was better than all other genotypes evaluated at multiplication. Beside, *Fadis -01* showed 25% yield advantage over the standard check, Hormat. On research field *Fadis-01* gave yield ranging from 3.8 to 4.1 ton ha⁻¹, whereas 3.5 to 4.0 tons ha⁻¹ on farmers field (Table 1).

Striga Reaction

Striga infestation data was recorded on field and at laboratory. Field data was collected at flowering and harvesting stage. The result showed that there were no significant difference in number of *Striga* both at flowering and harvesting (Table 2 and 3). Besides, there was no significant difference between the candidates and the standard check at field condition. The laboratory bioassay data of sorghum genotype 2006 MW 6031 showed the least value of MGD (maximum germination distance) and GR (germination rate) with 1.52 mm and 1.95%, respectively, followed by resistant check Hormat. The result revealed that there was no significant difference between *Fadis-01* (M-36121 X P-9403) and standard check (Hormat) in MGD and GR (Table 4).

Table 1. Agronomic and morphological characteristics of Fadis01 (M-36121 X P-9403)

Agronomic characters and descriptions of Fadis	
Variety name:	Fadis01(M-36121 X P-9403)
Adaptation area:	Fadis, Babile, Harari, Dire Dawa and other lowland sorghum growing areas
Altitude (masl):	<1700
Rainfall (mm):	400-700
Seeding rate (kg/ha):	8-10 kg for row spacing
	12-20 kg for broadcasting
Spacing (cm):	20cm Between rows
Planting date:	Mid June to early July
Fertilizer rate (kg/ha):	100 NPS all at planting
	50 UREA at knee height
Days to flowering:	74-79, mean 77 days
Days to maturity:	120 days
1000 seed weight (g):	30
Panicle appearance	Semi compact and erect
Plant height (cm):	143-180, mean 162
Seed color:	White(creamy color)
Grain yield (qt/ha):	Research field: 38-48
	Farmers field: 30-41
Tillering:	No tillers
Crop pest reaction	Tolerant/resistant to striga weed
Year of release:	2019
Breeder/ maintainer:	FARC/OARI

Table 2 Mean number of striga weed in multi-location trial and during different years at flowering stage

Genotypes	Fadis14	Babile14	Fadis 15	Babile 15	Fadis 16
2006 MW 6239	3(9.0)	3(8)	3(7) ^{ab}	28(812) ^b	0(0)
2006 MW 6031	5(22)	5(21)	3(14) ^{bc}	17(334) ^{ab}	0(0)
2006 MW 6044	2(4)	2(4)	3(10) ^{abc}	20(527) ^{ab}	0(1)
2006 MW 6001	4(13)	3(11)	2(4) ^a	14(258) ^{ab}	1(2)
2006 MW 6017(Fadis01)	5(30)	5(29)	2(6) ^{ab}	18(410) ^{ab}	1(2)
05 MW 6005	4(19)	4(18)	5(21) ^{bc}	12(165) ^a	1(3)
Hormat (St. check)	3(7)	3(7)	2(6) ^{ab}	12(159) ^a	0(1)
LSD	NS	NS	10.2	545	NS
CV	28.1	19.9	34.4	46.6	31.3

Key: Value in the parenthesis is the original values; numbers outside the parentheses are square root-transformed.

Table 3. Mean number of striga weed in multi-location trial and during different years at harvesting stage

Genotype/Check	Fadis14	Babile14	Fadis 15	Babile 15
2006 MW 6239	6(32)	1(2) ^a	4(13)	21(489)
2006 MW 6031	8(63)	6(33) ^d	4(18)	12(196)
2006 MW 6044	3(14)	3(7) ^c	3(12)	15(288)
2006 MW 6001	5(22)	2(3) ^{ab}	2(6)	10(134)
2006 MW 6017(Fadis01)	8(63)	2(5) ^{bc}	3(7)	13(252)
05 MW 6005	6(33)	2(4) ^{abc}	4(20)	10(128)
Hormat	5(20)	2(4) ^{abc}	3(7)	11(186)
LSD	NS	17.9	NS	NS
CV	18.2	22.5	35.1	47.8

Key: Value in the parenthesis is the original values; numbers outside the parentheses are square root-transformed.

Table 4. Mean comparison of Sorghum Genotypes for pre-attachment to *Striga* tested at bioassay lab during 2017 (2*2.5 cm near the host root)

Genotypes	Treats			
	MGD	GR	HIP	GI
2006 MW 6001	8.59 ^a	12.03 ^b	13.90 ^c	1.30 ^a
05 MW 6005	4.65 ^b	5.98 ^c	8.77 ^c	1.08 ^b
2006 MW 6017(Fadis-01)	1.48 ^c	29.33 ^a	23.07 ^{ab}	1.18 ^{ab}
2006 MW 6031	1.52 ^c	1.94 ^c	26.53 ^a	1.07 ^b
Hormat	0.83 ^c	4.96 ^c	14.67 ^{bc}	1.27 ^a
LSD (5%)	1.15	4.13	8.48	0.16
CV%	10.20	20.20	25.90	7.20

Key: MGD= Maximum Germination Distance of *Striga* from the host root; GR= Germination rate of *Striga*; HIP= Haustorial initiation percentage; GI= Germination index.

Stability performance

The Genotype and Genotype by Environment (GGE) biplot analysis revealed that the released variety Fadis01 (M-36121 X P-9403) fall

relatively close to the concentric circle near to average environment axis, suggesting its potential for wider adaptability with better grain yield performance (Fig 1).

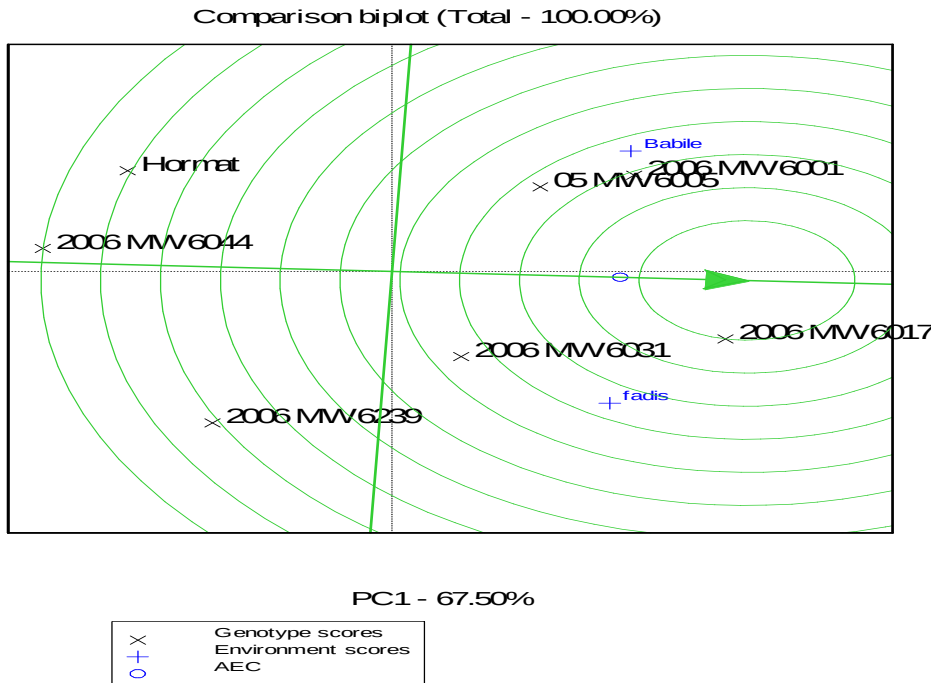


Fig 1: GGE biplot for grain yield kg ha⁻¹ of sorghum genotypes tested at two locations during 2006, 2007 and 2008 main cropping season

Adaptation

Fadis-01 is released for the lowlands of eastern Hararghe and similar agro-ecology receiving sufficient amount of rainfall (400mm-710mm) and altitude ranges of 1200-1710 m.a.s.l. The variety performs best with its full agronomic recommendations as presented in Appendix 3. The variety matures at early and consequently has capacity to escape the recurrent terminal drought stress prevalent in lowland areas of Eastern Ethiopia particularly, Fadis and Babile (Erer) and areas with similar agro-ecology in the country.

Conclusion

Fadis-01 is stable in its grain yield, moderately resistance to *Striga* and has good agronomic traits that make it suitable for production in its recommended domain of Eastern Hararghe and areas with similar agro-ecology. The variety has several desirable traits for wider production mainly due to its yield performance, good plant height and relatively good striga resistant.

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The Release and Registration of “Jitu” Tef [*Eragrostis tef* (Zucc.)] Variety

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Abstract

The name **Jitu** was given to tef [*Eragrostis tef* (Zucc.)] variety with the pedigree of DZ-01-256 which was developed by Bako Agricultural Research Center. **Jitu** was evaluated together with eleven pipelines genotypes and the standard check (Kena) and local check in regional variety trial at three locations (Arjo, Gedo and Shambu) for two consecutive years (2017 and 2018). The combined analysis of variance revealed highly significant ($p < 0.01$) difference among genotypes for plant height, panicle length, lodging % and grain yield (kg ha^{-1}) and significant ($p < 0.05$) for maturity date and shoot biomass. Genotype and Genotype by Environment interaction (GGE) biplot analysis revealed that **Jitu** (DZ-01-256) was the most stable and high yielding variety ($2309.22 \text{ kg ha}^{-1}$) with 25% yield advantage over the standard check, Kena ($1683.92 \text{ kg ha}^{-1}$), tolerant to lodging and thus was released in 2019 for the highlands of Arjo, Gedo, Shambu and similar agro-ecologies.

Keywords: Bi-plot, Genotype and Genotype by Environment Interaction (GGE), Tef (*Eragrostis tef* (Zucc.),

Introduction

Tef is among the major Ethiopian cereal crops grown on more than 3 million hectares annually (CSA, 2017), and serving as staple food 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). 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 variety for tef growing areas of western parts of the country

Varietal origin and evaluation

A total of 13 tef genotypes originally introduced from Ethiopian Institute of biodiversity and Debre Zeit Agricultural Research Center were evaluated at multi location (Shambu, Gedo and Arjo sub-sites) in 2016/17 and 2017/18 to identify stable high yield variety with other desirable traits. Accession DZ-01-256 (*Jitu*) gave the highest grain yield (2309.22 kg ha⁻¹) followed by accession DZ-01-1576 (2105.72 kg ha⁻¹). The standard check Kena gave 1683.92 kg ha⁻¹. Besides, *Jitu* showed stable performance over the six environments (year by location). The two genotypes, Acc. DZ-01-256 (*Jitu*) and DZ-01-513 gave above 10 percent yield advantage and preferable desirable traits other over the standard check and thus selected and evaluated against local & standard checks in variety Verification trial at Shambu, Gedo and Arjo sub site and on farmers field during 2018/19 (Table 1).

Agronomic and Morphological Characteristics

Jitu variety is well adapted to the highlands of Western Oromia and has an average plant height of 106 cm and maturity date of 120 days. The variety has high tillering capacity, high shoot biomass (13.80 t ha⁻¹) and grain yield (2309.22 kg ha⁻¹). The caryopsis color of the variety is cream-white with thousand seed weight of 0.26 gram and the leaves are staying green even after maturity period (Table 1, 2 and 3).

Yield Performance

The average grain yield of *Jitu* combined over locations and years were 2309.22 kg ha⁻¹, which is higher than Kena (standard check), 1683.92 kg ha⁻¹. Under research field, *Jitu* gave grain yield ranging from 2132.08-2532.5 kg ha⁻¹ while on farmers' field it ranges from 1961-2360 kg ha⁻¹.

Stability performance

Jitu variety showed stable yield performance across tested location over years. It performs best if it is produced with recommended fertilizer, seed rate and other recommended fertilizer rate in the recommended ecologies.

Conclusion

The new tef variety **Jitu** was released for its stable high grain yield and other desirable traits, wider adaptability, attractive seed color (cream white) and

tolerant to tef leaf rust. This new variety was released in May 2019 and recommended for production around Shambu, Gedo, Arjo and similar agro-ecologies.

Table 1. Agronomic & morphological characteristics of Jitu tef variety

Variety name:	Jitu (Acc. DZ-01-256)
Agronomic & morphological characteristics	
Adaptation area:	Shambu, Gedo, Arjo, and similar agro ecologies of western highlands.
Altitude (masl)	1800-22500
Rainfall (mm)	1800-2000
Seeding rate (kg/ha)	10 for row & 15 broad casting
Spacing (cm)	20cm Between rows
Planting date	Early July
Fertilizer rate	100 kg ha ⁻¹ DAP at planting & split application of 50 kg ha ⁻¹ UREA
Days to heading	80 days
Days to maturity	120 days
1000 seed weight (g)	0.26
Plant height (cm)	106
Seed color	cream white
Panicle color	yellowish at maturity
Crop pest reaction*	Tolerant to leaf rust
Grain yield (qt/ha)	
On farmers field	1961-2360 kg ha ⁻¹ .
On-station	2130-2530 kg ha ⁻¹ .
Year of release	2019
Breeder/ maintainer:	BARC/IQQO

Key: *Tolerant to major Tef diseases (Head smudge and Rust) & tolerant to acidic soils of Western highlands of Oromia Regional state.

Table 2. Mean grain yield(kg/ha) of tef genotypes across locations and years

Genotypes	Shambu		Gedo		Arjo		Mean	Yield advantage over st. check
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18		
DZ-01-1122	1800	1660	1822	1849	1618	1531	1713.39	
DZ-01-512	1840	1815	1958	1878	1609	1682	1796.83	
Local	1690	1538	1964	1549	1569	1477	1631.11	
DZ-01-2014	1786	1689	1957	1643	1403	1496	1662.31	
Kena (st check)	1694	1651	1822	1857	1517	1563	1683.92	
DZ-01-61	1745	1883	1840	1647	1758	1845	1786.33	
DZ-01-513	2170	2167	1847	2028	1982	1895	2014.75	19.65%
DZ-01-1715	1593	1634	1865	1523	1695	1768	1679.53	
DZ-01-1108	1822	1909	1770	1719	1643	1544	1734.39	
DZ-01-1576	2268	2462	1990	2079	1893	1943	2105.72	25.05%
DZ-01-383	1960	1782	1766	1793	1703	1851	1808.92	
DZ-01-256	2450	2615	2307	2219	2036	2228	2309.22	37.13%
DZ-01-1118	1810	1869	1660	1681	1237	1654	1651.83	
Mean	1755.07	1897.99	1889.79	1789.49	1306.49	1721.205	1813.71	
CV%	9.24	17.3	13.7	11.5	11.3	18.7		
F test	**	**	**	**	**	**		
LSD 0.05	240	224	178	167	174	189		

Table 3. Mean grain yield and agronomic performance of 13 tef genotypes tested in regional variety trial combined over three locations for 2016/17 and 2017/18

Entry No.	Genotypes	PH (cm)	MD	PL (cm)	LOD%	ET	SBM (t/ha)	GY kg/ha
1	DZ-01-1122	88.33	116.67	28.27	43.33	6.20	10.60	1713.39
2	DZ-01-512	90.00	117.67	25.33	31.67	7.20	11.33	1796.83
3	Local	74.33	117.67	22.00	86.67	6.80	10.17	1631.11
4	DZ-01-2014	88.33	120.00	26.00	31.67	6.80	9.33	1662.31
5	Kena (st check)	88.00	117.67	23.73	86.00	6.13	9.33	1683.92
6	DZ-01-61	81.67	118.00	25.47	43.33	7.07	10.75	1786.33
7	DZ-01-513	96.67	117.00	30.60	31.67	6.07	10.50	2014.75
8	DZ-01-1715	81.00	119.33	23.07	33.33	6.4	9.00	1679.53
9	DZ-01-1108	92.00	120.67	30.87	35.00	6.93	10.16	1734.39
10	DZ-01-1576	86.67	118.67	31.07	40.00	6.47	9.30	2105.72
11	DZ-01-383	86.00	118.00	29.6	31.67	6.67	11.08	1808.92
12	DZ-01-256	106.00	119.67	37.53	31.33	6.87	13.80	2309.22
13	DZ-01-1118	78.00	119.67	22.13	38.33	6.47	12.33	1651.83
Mean		87.46	118.51	27.36	44.49	6.62	8.41	1813.71
CV %		9.09	11.19	10.35	23.30	13.12	11.82	13.24
F test		**	*	**	**	NS	*	**
LSD		1.34	2.38	4.77	9.85	1.46	0.44	2.4

Note: PH = plant height(cm), MD = maturity date, PL = panicle length (cm), LOD =lodging %, ET = effective tiller, SBM(kg) = shoot bio-mass, GY = grain yield(kg)

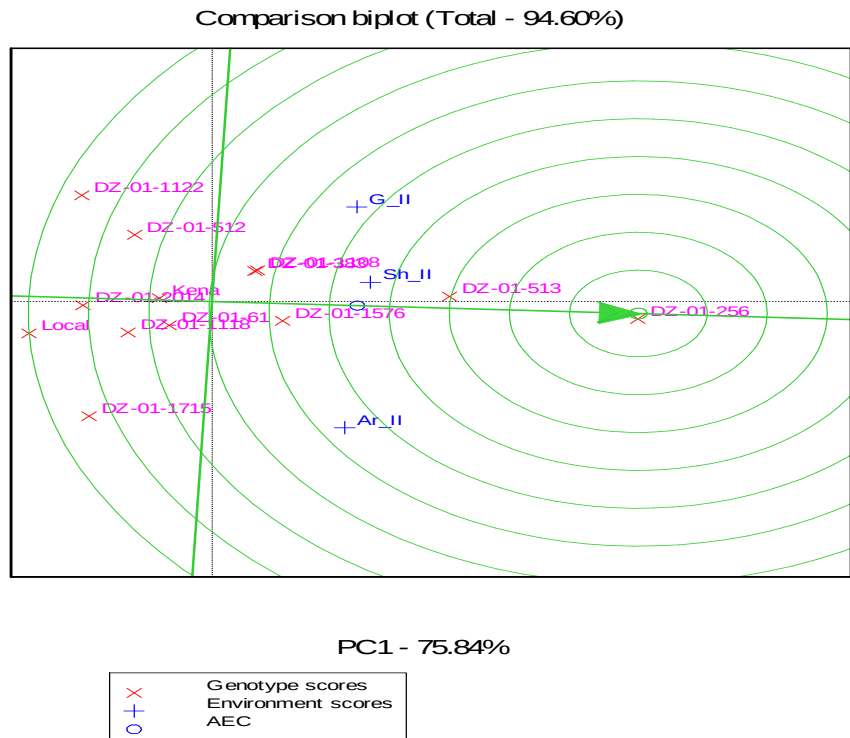


Fig.1 GGE bi-plot for stability test among tef genotypes

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