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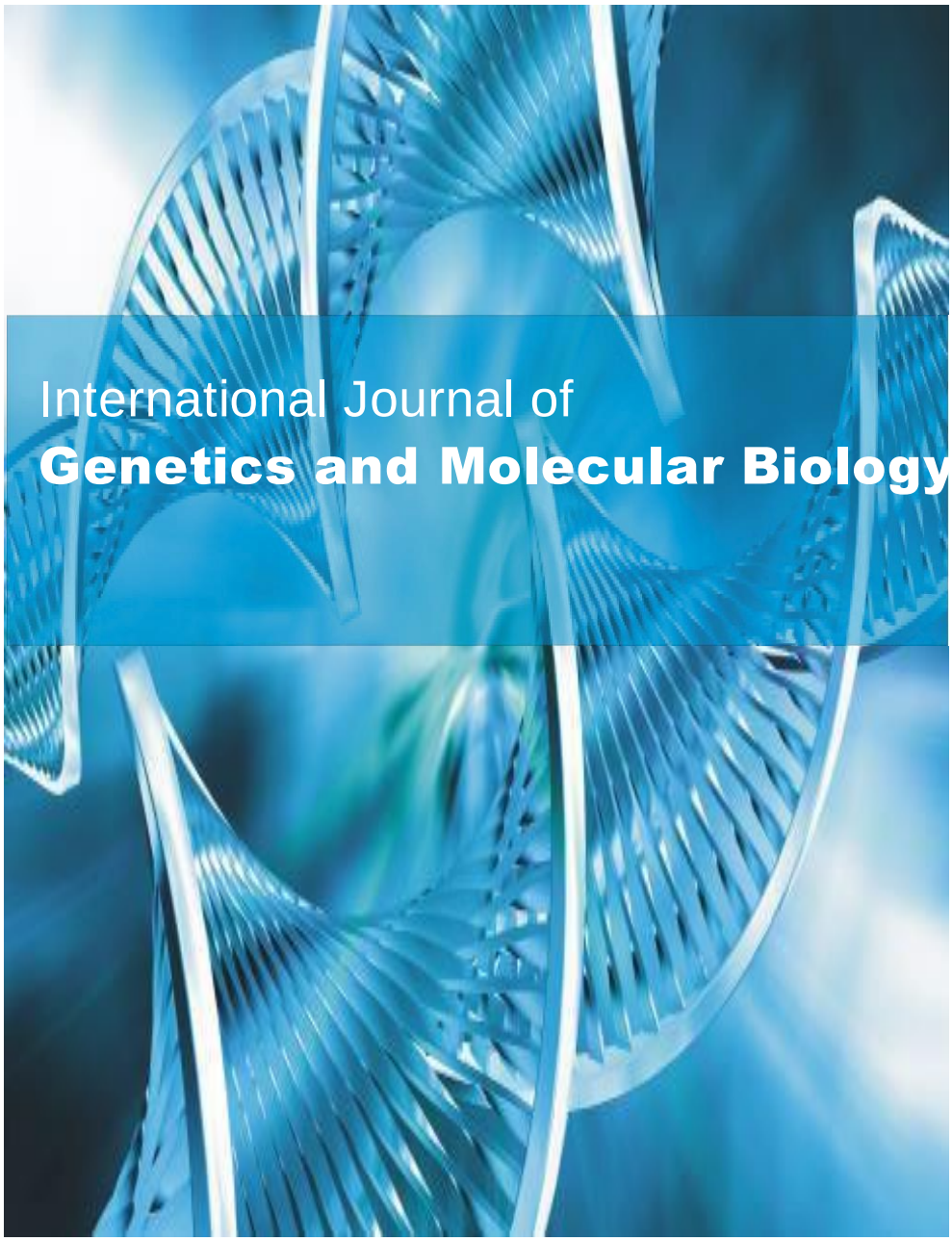

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Full Length Research Paper

Sequencing of S_5 gene in autotetraploid rice *japonica* and *indica* to overcome F_1 hybrids embryo sac sterility

Fimanekeni Ndaitavela Shivute[1,2,3,4,5*], Nabieu Kamara[1,2,3,4,6] and Xiang-Dong Liu[1,2,3,4,]

[1]State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou 510642, China.
[2]Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou 510642, China.
[3]Guangdong Provincial Key Laboratory of Plant Molecular Breeding, South China Agricultural University, Guangzhou 510642, China.
[4]College of Agriculture, South China Agricultural University, Guangzhou 510642, China.
[5]Researcher, Multi-disciplinary Research Services, University of Namibia, Namibia.
[6]Sierra Leone Agricultural Research Institute (SLARI), Freetown PMB 1313, Sierra Leone.

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Autotetraploid rice is a new germplasm developed through diploid chromosome doubling. Hybrids developed by *indica* autotetraploid rice crossed by *japonica* autotetraploid rice has clear biological advantage on F_1, causing widespread concerns for special research evolution to evaluate and utilize F_1 hybrid vigor. However, the widespread fertility of F_1 *indica* and *japonica* autotetraploid rice is low, which makes it difficult to direct utilization of F_1 vigor. In diploid *indica* and *japonica* rice, the fertility of F_1 hybrids is also proved to be low. Embryo sac infertility is known to be one of the most important reasons for hybrid sterility and many studies has indicated that the primary cause of F1 sterility was abortion of the embryo sac, which was identified by the genotype of the S5 gene on the chromosome. Previous studies have cloned S_5 based on common wild rice and cultivated rice and S_5 sequence was obtained and studied. In this paper, typical sequence of different materials of S_5 *japonica* and *indica* rice group of autotetraploid hybrids observed with whole-mount eosin B-staining confocal laser scanning microscopy WE-CLSM was utilized, to overcome F_1 hybrids embryo sac sterility.

Key words: rice (*Oryza sativa* L.); autotetraploid; hybrid sterility; S_5 gene; embryo sac fertility.

INTRODUCTION

Food security, which is the condition of having enough food to provide adequate nutrition for a healthy life, is a critical issue in the world (Chen et al., 1994). About 3 billion people, nearly half the world's population, depend on rice for survival (Kanawapee et al., 2011) and in Asia as a whole, much of the population consumes rice in every meal (Maclean et al., 2002). The genus Oryza originated many years ago and different species got distributed into different continents.

Genus *Oryza sativa*, the Asian cultivated rice is grown

*Corresponding author. E-mail: fshivute@unam.na.

all over the world (Khush, 1997), while African cultivated rice, *O. glaberrima* is grown on a small scale in West Africa (Mmbando, 2022). The cultivated species originated from a common ancestor with AA genome. Perennial and annual ancestors of *O. sativa* are *O. rufipogon* and *O. nivara* and those of *O. glaberrima* are *O. longistaminata/, O. breviligulata* and *O. glaberrima* are domesticated in Niger River delta (Sangeetha et al., 2020). Rice is the most important crop to millions of small farmers who grow it on millions of hectares throughout the region, and to the many landless workers who derive income from working on these farms (Ganesan et al., 2022; Shennan et al., 2022). In the future, it is imperative that rice production continue to grow rapidly as the population upsurge, and therefore increased yield became a major concern for rice scientists.

Oryza sativa, commonly known as Asian rice is classified into two main subspecies, *indica* and *japonica*. Interspecific (*indica* × *japonica*) hybrids have great biological superiority (Tong et al., 2011; Zheng et al., 2020; Ouyang et al., 2022) and among all these interspecific hybrids are highly resistant against insect, pest and diseases (Gaikwad et al., 2021; Cordero-Lara, 2020) with enhanced drought tolerance (Kang and Futakuchi, 2019; Jia et al., 2022) as well as high biomass production (Wendel, 2000; Li et al., 2022, 2012). However, these hybrids seed setting rate is low (Zheng et al., 2020; Huang et al., 2021; Kim et al., 2022). In order to create hybrid rice, researchers have been very interested in the significant hybrid vigor between the indica (O. sativa ssp. indica) and japonica (O. sativa ssp. japonica) subspecies of the Asian farmed rice (O. sativa L.). The partial sterility that frequently occurs in indica-japonica crossings is a significant obstacle to the production of such inter-sub specific hybrids (Kato et al., 1928, Sweigart et al., 2019; Zhang, 2020; Ouyang et al., 2022). Similar research revealed that indica-japonica hybrids' fertility varied greatly, from totally fertile to nearly completely infertile (Fang et al., 2019; Wang et al., 2020; Ouyang et al., 2022) while the majority of inter-sub specific hybrids have markedly decreased fertility (Oka, 1974; Zhang et al., 1997; Li et al., 2020; Kim et al., 2022).

Poor fertility is the main barrier for utilizing heterosis between the two rice (*O. sativa* L.) subspecies, *indica* and *japonica* and the development of autotetraploid hybrids ($2n = 4x = 48$) has been advised as a new method for increasing heterosis in hybrid rice (Chen et al., 2019; Ghaleb et al., 2020; Rao et al., 2022). An autotetraploid *indica/japonica* hybrid combines the advantages of polyploidy and heterosis between *indica and japonica* (Hu et al., 2010; Yu et al., 2021a; Huang and Huang, 2022; Rao et al., 2022) and realized to have great potential to increase the yield of rice (He et al., 2011; Ghouri et al., 2019; Chen et al., 2021; Ku et al., 2022). Furthermore, autotetraploid *indica/japonica* hybrids hold more nutrition value than diploid rice (Zhiyong et al., 1987 and He et al., 2011; Chen et al., 2021, 2022; Ghouri et al., 2023) and therefore said to have stronger potential vigor in rice breeding than diploid rice does (Shahid et al., 2011; Rout et al., 2020; Wu et al., 2020; Chen et al., 2022). Hybrid sterility between two rice subspecies may be overcome by using tetraploid lines followed by intensive selection (Chen et al., 2021; Huang et al., 2022). Also, the gigantic features of the autotetraploid hybrids may establish a plant structure able to support the higher yield (Shen et al., 2022; Zeng et al., 2023). However, the utilization of strong heterosis in the F_1 hybrids between the two subspecies has been difficult because of partial or complete sterility in the hybrids (Shahid et al., 2013; Yu et al., 2021a; Ouyang et al., 2022).

Though, heterosis utilization has been successful in many crops (Liu et al., 1998; Xiao et al., 2021; Landge et al., 2022), not much success on autotetraploid rice as most results were based on diploids crops. According to He et al. (2011) using polyploidy meiosis stability (PMeS) line as a parent improves embryo development and the seed set rate of a tetraploid rice hybrid. Moreover, polyploidy is widely accepted to play an important role in the evolution and breeding of plant species (Udall and Wendel, 2006; Cheng et al., 2022; Mangena, 2023), but nonetheless, a low seed set rate significantly hindered the development of polyploidy rice breeding (He et al., 2011; Xiong et al., 2019; Zhang et al., 2019, Chen et al., 2021; Huang et al., 2022). Consequently, there is an urgent need to overcome sterility of interspecific hybrids of autotetraploid rice in order to utilize its great genetic competence and explore its hybrid vigor.

Embryo sac fertility and pollen fertility are the most important factors which affect the seed setting rate in autotetraploid rice (Shahid et al., 2010; Li et al., 2020; Kamara et al., 2021; Ku et al., 2022, Kamara et al., 2022) and additionally lead to indica sterility hybrid japonicas (Song et al., 2005; Mi et al., 2019; Rout et al., 2020) since they both significantly affect hybrid fertility.

According to the well-known theory put forth by Ikehashi and Araki (1986), F1 sterility was mostly brought on by embryo sac abortion, which was determined by the genotype of S_5 located in chromosome 6, and *indica* was $S_5^iS_5^i$ in genotype as well as *japonica* $S_5^jS_5^j$ (Yang et al., 2009; Zhang et al., 2020; Seo et al., 2020; Kallugudi et al., 2022) hence The S_5 locus on chromosome 6 became the primary genetic cause of embryo sac sterility due to several allelic interactions (Lee et al., 2021; Zhang, 2021; Ouyang et al., 2022). Nevertheless, hybrid fertility was decreased as a result of interactions between the indica and japonica alleles at each of the loci (Xie et al., 2019; Guo et al., 2022; Kallugudi et al., 2022). Yang further explained that because to gene interactions, the indica and japonica parent types' S5iS5j hybrids were also infertile S_5^i and S_5^j.

Wide-compatibility varieties (WCVs), a special subset of rice germplasm, have made intersub specific heterosis more useful (Ikehashi and Araki, 1986; Awad-Allah, 2020;

Zhang, 2020; Kallugudi et al., 2022). When crossed to indica or japonica subspecies, WCVs can overcome obstacles to reproduction and result in fertile hybrids. Hybrids between indica and japonica parent lines that carry the S_5n (wide-compatible gene) would have high or normal fertility regardless of whether they have the S_5nS_5i or S_5nS_5j genotype (Li et al., 2011; Ghaleb et al., 2020; Guo et al., 2022). Wide-compatibility varieties (WCVs) allowed the reproductive barrier between the indica and japonica subspecies to be breached and gave rice breeding programs the opportunity to create inter-sub specific hybrids.

Progress has been made by previous studies for S_5^n gene sequencing especially in diploid rice and their results indicated that widely compatible kinds ($S_5^nS_5^n$) can produce fruitful hybrids when bred with indica or japonica varieties (Kinoshita, 1995; Yu et al., 2021; Ghaleb et al., 2020; Vernet et al., 2022). Rice geneticists also cloned S_5^n and found that *the two nucleotide differences between S_5i and S_5j resulted in two amino acid substitutions in the relevant protein and intersub specific hybrid sterility* (Chen et al., 2008; Lu et al., 2020: Rout et al., 2020; Yu et al., 2021b).

MATERIALS AND METHODS

Forty-nine autotetraploid rice lines which were planted in the South China Agricultural University's experimental farm was used in this experiment and different hybrids made by *indica and japonica* autotetraploid varieties which were planted at the same field were also used, with typical hybrids (*indica* and *japonica*) used as control.

Sequencing of S_5 gene in autotetraploid rice

The results of forty-nine autotetraploid rice lines used in this paper were amplified and sequenced by the Regional Genetic and Breeding Laboratory of South China Agricultural University, College of Agriculture, Guangzhou, Guangdong. The laboratory primers were synthesized by Shanghai Biological Engineering Technology Services Limited Company and these primers were designed on the basis of the sequences of Nipponbare ($S_5^jS_5^j$) and 02428 ($S_5^nS_5^n$) cultivars. This paper used the sequenced results.

Embryo-sac fertility observation

Zeng et al. (2007) used whole-mount eosin B-staining confocal laser scanning microscopy (WE-CLSM) to investigate the embryo-sac structure. 100 to 150 spikelets with developed embryo sacs were taken from each plant after blooming and fixed in FAA (formaldehyde: acetic acid: 50% ethanol = 5:6:89) for at least 24 h. The florets were cleaned in 50% ethanol before being kept at 4°C in 70% ethanol. Under a binocular dissecting microscope, the ovaries were dissected in a Petri dish and successively hydrated in 50% ethanol, 30% ethanol, and distilled water. The samples will undergo a 20 min pretreatment in 2% aluminum potassium sulphate before being stained for 12 h at room temperature with a 10 mg/L solution of eosin B diluted in 4% sucrose. The samples underwent a 20-min post-treatment in 2% aluminum potassium sulphate. The samples were dehydrated using a series of ethanol solutions (30, 50, 70, 90 and 100%) after being rinsed three times with distilled water. The dehydrated samples were then transferred to a 1:1 mixture of absolute ethanol and methyl salicylate for 1 h, followed by at least 1 h of clearing in pure methyl salicylate solution. Finally, a Leica SPE laser scanning confocal microscope was used to scan the samples.

Seed setting observation

The Shi et al. (2009) approach was used to calculate the seed-set rate. In a nutshell, the average seed dry weight of the harvested individuals was used to calculate seed yield (SY). For each replication sample, 1000 fully formed seeds were used to calculate the seed weight (SW). The average number of well-filled seeds from 100 fully formed pods, which were taken from the principal branch in the centre of the harvested individuals, was tallied to determine the seed number (SN). The number of normally grown pods on each harvested person was known as the pod number (PN). Each harvested individual's plant height (PH) was calculated by measuring it from the stem's base to the tip of the main shoot. The number of operational primary branches (BN) was recorded.

RESULTS

S_5 gene sequence variation

According to the information supplied, forty-nine autotetraploid rice lines sequenced carried S_5 gene sequence. Ten haplotypes carried S_5^n gene while thirty-nine haplotypes carried either S_5^i or S_5^j gene. Figure 1 shows the sequence variations of S_5 loci among 49 autotetraploid rice lines while Figure 2 shows S_5 gene variation analysis among 10 autotetraploid rice varieties carrying S_5^n gene. ▂, exon; –intron; *, base position (the first transcription starting point of 02428 is + 1).

Embryo sac fertility and seed setting of *indica* and *japonica* autotetraploid hybrids

As it can be seen from Table 2, a big difference is observed in embryo sac fertility of autotetraploid parents among different materials, embryo sac fertility of 4 materials (T432, T440, T455 and T434) were more than 80%, and low embryo sac fertility rate below 70% were observed in 3 materials (T412, T416 and T424), parent with the highest embryo sac percentage was T432 with 92.25%, with the lowest being T412 with 31.32%. The seed setting results shows that all parents observed were below 70% except T440 which has a seed setting rate of 72.90% and it is the highest among all the observed parents. Embryo sac fertility decreases gradually especially with parent T432 which has embryo sac fertility of 92.25%, but very low seed setting rate of 2.53%, this result may be due to low pollen fertility of these material. Other rice parents like T412 has low embryo sac fertility of only 31.32%, but high seed setting rate of 69.90%, this result may be due to the influences in embryo sac fertility

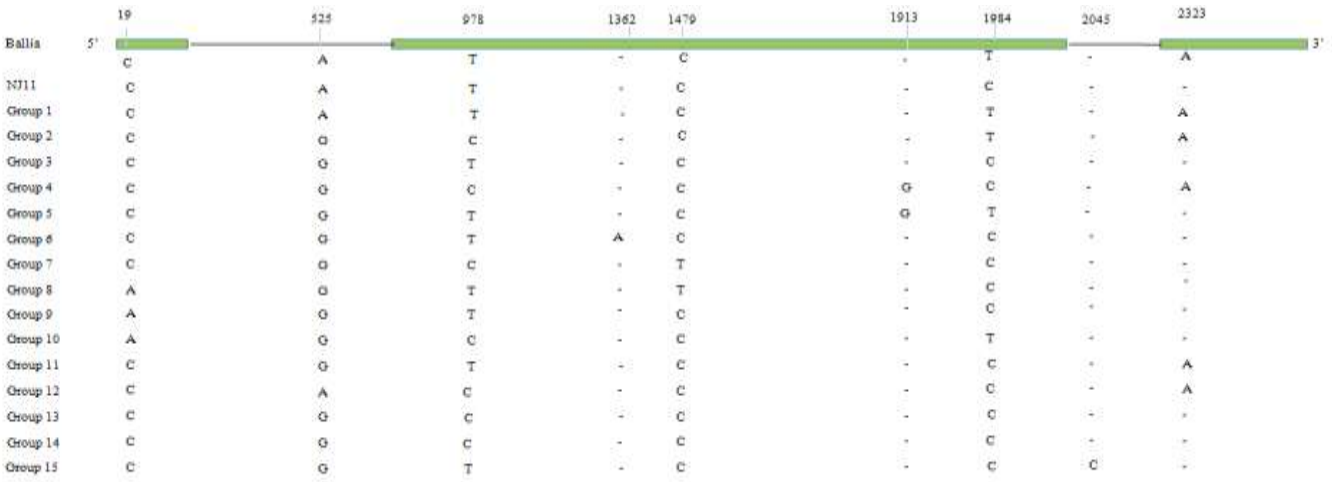

Figure 1. Sequence variations of S_5 loci among 49 autotetraploid rice lines.
Source: Supplied by the Genetic and Breeding Laboratory of South China Agricultural University, College of Agriculture, Guangzhou, Guangdong Province, China.

Figure 2. S_5 gene variation analysis among 10 autotetraploid rice varieties carrying S_5^n gene. ▄, exon; –intron; *, base position (the first transcription starting point of 02428 is + 1).
Source: Supplied by the Genetic and Breeding Laboratory of South China Agricultural University, College of Agriculture, Guangzhou, Guangdong Province, China.

and seed rates from different plant genetic characteristics of this material which may not be stable enough and thus has led offspring separation and differences when it comes to the genetic characteristics and materials instability, which might have caused embryo sac fertility and seed setting deviation. Therefore, in autotetraploid rice genetic research, there is a great need of research expansion especially to the community, as community outreach and research in order to obtain more accurate results.

Moreover, in the total number of 9 hybrids which was observed for embryo sac fertility, only hybrid (T453 x T434) has less embryo sac fertility rate which is less than 80% (70.33%), the remaining 8 hybrids has high embryo sac fertility rate which is higher than 80%, the average embryo sac fertility rate stands at 89.12%. However, this results found it out that there was a significant different between seed setting rate and embryo sac fertility rate of the some materials with the same parent materials like T424 x T440 which has very high embryo sac fertility of 80.23% but very low seed setting rate of 3.49%, this result might also be due to low pollen fertility. Typical *indica-japonica* (without S_5^n) hybrids (T453 × T434) as well as *japonica-japonica* (without *indica* and S_5^n) hybrids (T424 X T440) generated embryo sacs with a very poor fertility, and many malformed embryo sacs were seen.

Abnormal embryo sacs that either lack an egg apparatus or have polar nuclei that are not properly positioned, normally prevents fertilization to occur, resulting in sterile embryo sacs. As can be seen from Table 3, the total percentage abortion rate of embryo sac rate for both parents and hybrid F_1 generation materials are different from each other with different types of abnormality expressed in different percentages. However, in all types of abnormality, embryo sac degradation (embryo SAC degeneration) is the most common type, except material T432 (parent) and T432 x T416 (hybrid) which remained with zero percentage embryo sac degeneration. Frequent abnormality appeared in all other materials especially embryo sac without egg apparatus, small embryo sac (Abnormal Small embryo sac) and abnormal polar nuclei (Embryo sac with abnormal polar nuclei). The remained three other types of embryo sac abortion were relatively rare. Among all, only 4 materials (T416, T432, T455 and T424 x T425) 4 materials have a shown relatively small amount of Embryo sac without egg Apparatus. Very small percentage of Embryo sac without female germ unit were shown T412, T424, T455 x T424 6

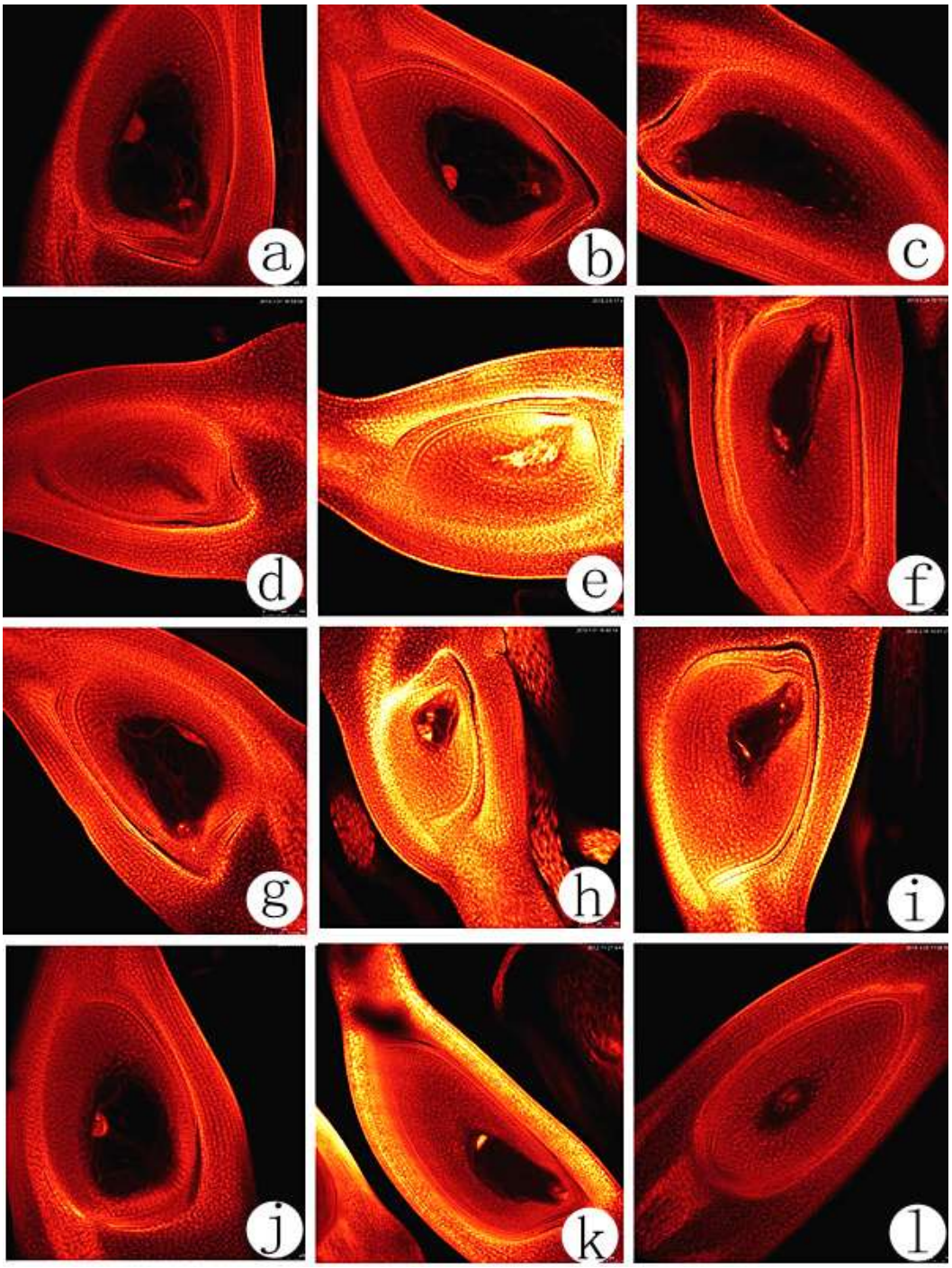

Figure 3. Abnormal embryo sac structures of *indica-japonica* hybrids $F_{1.}$ (a) and (b) normal embryo (With all normal egg, polar nuclei and antipodal cells positioned at the right positions) (c) Normal embryo sac after fertilization (d) and (e) Embryo sac degradation, (f) and (g) Embryo sac without an egg apparatus, (h) and (i) Abnormal small embryo sacs, (j) Embryo sac without female germ unit, (k) Embryo sac with abnormal position of polar nuclei and (l) Others.
Source: Authors

while T440, T424 x T43 and T453 x T434 have relatively small percentages of other types of abnormal embryo.

Abnormal embryo sacs included embryo sac degeneration (Figure 3d and e), embryo sac without egg apparatus (Figure 3e) abnormal small embryo sac (Figure 3g), embryo sac without female germ unit (Figure 3j)

Table 1. Names and types of 49 autotetraploid lines/cultivars.

Material type	Material name
Indica	T41, T42, T44, T410, T413, T417, T419, T420, T421, T425, T426, T428, T429, T430, T442, T442-1, T445, T447, T448, T451, T452, T453, T455, T457, T461, T463, T465, T467, T469, T473 and T479
Japonica	T43, T45, T46, T48, T49, T415, T422, T423, T431, T432, T434, T435, T437, T438, T443, T444, T450 and T456

Thirty-one of these materials are *indica* while eighteen materials are *japonica.*
Source: Authors

Table 2. Embryo sac fertility and seed set of parents and their testcross F_1.

Name of parents and hybrids	Embryo sac fertility (%)	Seed set (%)
T412	31.32	69.90
T416	64.09	18.58
T424	57.36	20.27
T432	92.25	2.53
T440	86.86	72.90
T455	81.39	43.05
T434	90.89	49.78
T424 x T43	95.10	73.76
T455 x T424	88.42	68.81
T424 x T425	82.83	55.88
T432 x T416	96.29	57.71
T416 x T434	97.80	39.72
T435 x T416	98.27	52.66
T432 x T455	92.81	64.20
T424 x T440	80.23	3.49
T453 x T434	70.33	12.87

Source: Authors

and embryo sac with abnormal polar nuclei (Figure 3k). These embryo sacs can't fertilize regularly because they either lack an egg apparatus or have polar nuclei that are positioned incorrectly, leading to sterile embryo sacs. Other abnormal kinds, which were classified as other abnormal types because their frequencies were low, were also discovered in addition to the principal types of embryo sac defects stated above (Figure 3l).

Effects of different sequences of S_5 on embryo sac fertility of intersubspecific hybrids in autotetraploid rice

Table 4 illustrates typical indica-japonica (without S5n) hybrids (T453 T434), while hybrids between japonica and japonica (without indica and S5n) (T424 X T440) produced a variety of malformed embryo sacs and had very poor embryo sac fertility. Furthermore, typical *indica-japonica* (without S_5^n gene produced the least seed setting percentages (22.02%) as shown in Table 3, while in the same table, varieties with S_5^n gene come out with the highest seed setting percentages (69.32%).

Genetic variations are clearly seen in Table 4. In general, there are many genes that controls seed set and S_5^n is one of those gene, which mostly control embryo sac fertility and thus can be the reason why some materials without S_5^n have high seed set rate as compared to those with S_5^n gene, simply because some other genes were stronger and could be expressed more frequently. The highest F_1 generation seed setting rate S_5^j X S_5^n were 66.14 and 61.81% respectively, the highest seed set rate percentages of S_5^j X S_5^n is 69.32% with the least percentage being 22.02% for typical indica-*japonica*. Standard indica and japonica cultivars' embryo sac fertility and seed set rate considerably increased when they were crossed with S5n-harboring cultivars (Table 1 and 3). Indica-japonica cultivars and cultivars with the exon 2 10 bp deletion were crossed to create some testcross F1 hybrids, whose average embryo sac fertility was similarly quite high (69.32%). When crossed with normal cultivars, all S5n-containing cultivars or accessions showed high embryo sac fertility, as shown in Table 1 (T435 x T416) 98.27%, demonstrating that the

Table 3. Types of abnormal mature embryo sacs in different rice materials (%).

Material	Total abortion rate of embryo sac	Embryo sac degeneration	Abnormal small embryo sac	Embryo sac with abnormal polar nuclei	Embryo sac without egg apparatus	Embryo sac without female germ unit	Other types of abnormal embryo sac
T412	68.68	39.20	24.41	4 .67	0.00	0.40	0.00
T416	35.91	18.93	11.39	4.89	0.70	0.00	0.00
T424	42.64	16.00	0.00	24.60	0.00	2.04	0.00
T432	7.75	0.00	5.52	1.21	1.02	0.00	0.00
T440	13.14	9.43	0.00	2.71	0.00	0.00	1.00
T455	18.61	4.12	7.20	4.52	2.77	0.00	0.00
T434	9.11	2.43	3.98	2.70	0.00	0.00	0.00
T424 x T43	4.90	2.48	1.72	0.00	0.00	0.00	0.70
T455 x T424	11.58	6.49	3.12	0.39	0.00	1.58	0.00
T424 x T425	17.17	4.95	0.90	10.32	1.00	0.00	0.00
T432 x T416	3.71	2.10	0.00	1.61	0.00	0.00	0.00
T416 x T434	2.20	0.70	1.10	0.00	0.00	0.40	0.00
T435 x T416	1.73	1.33	0.00	0.40	0.00	0.00	0.00
T432 x T455	7.19	0.00	3.42	2.19	0.00	1.58	0.00
T424 x T440	19.77	11.37	4.65	1.02	0.00	2.73	0.00
T453 x T434	29.67	12.67	5.92	9.38	0.00	0.00	1.70

Source: Authors

Table 4. Genotypic analysis of selected hybrid F_1 generation with seed setting.

Gene type	Hybrids	Seed set (%)
S_5^j X S_5^i	T433 X T469	59.08
	T433 X T458	46.56
	T434 X T452	22.02
	T438 X T413	46.94
	T433 X T443	37.50
S_5^j X S_5^n	T432 X T45	28.32
	T434 X T45	61.81
	T434 X T443	66.14
	T435 X T45	35.55
S_5^i X S_5^n	T44 X T45	69.32

Source: Authors

S5nsequence variation has no effect on S5n's capacity to overcome embryo sac sterility.

DISCUSSION

The reproductive processes, which comprise pollen and embryo sac formation, fertilization, embryogenesis, and endosperm, are significantly responsible for the potential for normal seed set (Brukhin and Albertini, 2021; Underwood et al., 2022; Hu et al., 2023). Plant geneticists and breeders (He et al., 2019; Lu et al., 2020; Mohapatra and Sahu, 2021) examined the ovaries of autotetraploid inter-sub specific hybrids at 1 day and 7 days post-pollination and discovered that some embryo sacs with normal structure did not undergo fertilization, and they also noticed that some ovaries had embryo and endosperm development that was delayed. They also found low seed set, which is consistent with the findings of this article, proving that factors other than embryo sac and pollen fertility also affected seed set. It is advised to conduct more research to identify additional variables that can affect seed planting and embryo sac fertility.

Conclusions

When compared to indica-japonica hybrids without the S5n gene, the embryo sac sterility of these hybrids has improved dramatically. Even though there were no appreciable variations in the materials with opposing sequences, this blatantly demonstrated that S_5n can be employed as a tool to overcome embryo sterility in indica-japonica hybrids while tortuously demonstrating that S_5n is a non-functional gene.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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Genetic variability, heritability and genetic advance among yield and yield related traits of advanced Tef [*Eragrostis tef (Zucc.)* Trotter] breeding lines

Girma Ashe[1]*, Solomon Chanyalew[1] and Bulti Tesso[2]

[1]Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, P. O. Box: 2003, Addis Ababa, Ethiopia.
[2]Haramaya University, Haramaya, Ethiopia.

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In Ethiopia, tef is one of the most significant crops that are grown extensively as a staple cereal crop. The evaluation of genetic variability in crop species is one of the key activities in plant breeding, which supports in the creation of breeding strategies to meet a diversify objectives. A field experiment was therefore conducted to determine genetic variability, heritability, and genetic advanced for yield and yield-related traits of tef genotypes. The experiment was laid out in 7x7 simple lattice designs at two locations (Bishoftu and Akaki) in central Ethiopia during the 2021/22 main cropping season. For the majority of the parameters, the combined analysis of variance over locations revealed significance differences in location, genotype, and genotype x location interactions. The genotypic coefficient of variation (GCV) ranged from 2.96% for the number of primary panicle branches per main shoot to 15.82% for days to physiological maturity, while the phenotypic coefficients of variation (PCV) ranged from 3.62% for days to physiological maturity to 18.42% for the number of primary panicle branches per main shoot. Genetic advanced as a percentage of mean ranges from 2.43% (number of total tillers per plant) to 28.03% (number of primary panicle branches per main shoot) and heritability in the broadest sense ranges from 14% (number of total tillers per plant) to 88.67% (day to heading), respectively. High heritability coupled with high genetic advance as percentage of mean was recorded for the number of spikelets per panicle, number of primary panicle branches per main shoot and panicle length. Generally, the variation observed among the tested genotypes confirmed the possibility of improving tef genotypes for better yield through selection and hybridization.

Key words: Genotypic coefficient of variation, genetic advance, genetic variability, heritability, phenotypic coefficient of variation.

INTRODUCTION

Tef [*Eragrostis tef (Zucc.)* Trotter] (2n =4x =40] belongs to the family Poaceae and the genus *Eragrostis*. In Ethiopia, tef is the most significant crop grown for a different use. Because of the tef grain's nutritional and health advantages, as well as the fact that it doesn't contain gluten, the substance that causes celiac disease,

*Corresponding author. E-mail: girmaashe430@gmail.com.

the production of tef grain is gaining popularity on a global scale (Spaenij-Dekking et al., 2005; Hopman et al., 2008; Bergamo et al., 2011). In terms of production and consumption, it is the most significant staple cereal crop that thrives in various climatic and soil environments (Neela and Solomon, 2018).

Tef is a cereal crop that can grow in several of ecological conditions, from below the sea level to 3000 m above sea
level (m. a. s. l.). More over 7.1 million smallholder farmers produced tef on 3.1 million hectares of land in 2019/20, making up around 24.1% of the total area used for grain cultivation in the country. In Ethiopia, 40% of smallholder farmer households cultivated tef, which accounts for 17% of all grain production (CSA, 2020) and is the most significant economically important crop. Amhara and Oromia are the two main tef producing regions in Ethiopia, and together they account for 85.3% of the country's land area and 87.2% of its production, with an overall average productivity of 1.85 t/ha (CSA, 2020).

According to Allard (1960) and Falconer and Mackay (1996), variability is the occurrence of differences between individuals as a result of their genetic make-up and/or the environment in which they are raised. Differences in character expression between two individuals would be caused by genetic control if it were possible to measure these differences in an environment that was the same for both individuals (Falconer and Mackay, 1996).

For the crop to be further improved, understanding the degree and pattern of genetic variability present in a population is crucial. Understanding genetic variation is critical for improving yield and its components in any crop, as observable variability results from a combination of genetic, environmental, and numerous interactions between genes and environments. Thus, there is a need to evaluate the available genotypes for genetic variability and identify the best performing genotypes for future use in the breeding program. Therefore, the present study was, conducted, to assess genetic variability, heritability, and genetic advance for yield and yield-related traits in advanced tef breeding lines.

MATERIALS AND METHODS

Descriptions of experimental locations

The field experiment was carried out at the Debre Zeit Agricultural Research Center (DZARC) main station (Bishoftu) and the Akaki sub-station during the 2021/22 main cropping season. Bishoftu is located at (8° 44' N, 38° 58' E, and 1900 m.a.s.l) whereas Akaki at (8^{o} 53' N, 38° 58' E, and 2400 m.a.s.l) latitude, longitude, and altitude, respectively. The two locations are characterized by a moist tropical climate and experience a long rainy season extending from June to September. Bishoftu receives a mean annual rainfall of 832 mm during the main growing season, with maximum and minimum mean annual temperatures of 24.3 and 8.9°C, respectively. In contrast, Akaki often receives annual total rainfall of 1254 mm with maximum and minimum mean annual temperatures of 30 and 10°C, respectively. The experimental field at both locations is characterized by heavy black soil (vertisoil) with a very high moisture retention capacity.

Experimental plant materials

The experimental tef plant materials were obtained from Debre Zeit Agricultural Research Center of the National Tef Breeding Program. Forty-nine genotypes (48 advanced lines and 1 standard check (Dagim)) were used in the experiment. Table 1 shows the list and description of tef genotypes used for the study.

Experimental design, layout and management

The experiment was set up using a 7x7 simple lattice design with two replications. Each experimental plot measured 2 m^2 (1 m × 2 m) and had five rows that were 20 cm apart. Distances between incomplete blocks and between plots within incomplete blocks were 1.5 and 1 m, respectively. Within each replication, the genotypes were distributed randomly to plots. All additional crop management practices and recommendations were uniformly implemented to all genotypes as recommended for the crop.

Data collection and analysis

Days to 50% heading, days to 90% physiological maturity, grain filling period, plant height, panicle length, peduncle length, culm length, number of spikelets per panicle, number of primary panicle branches per main shoot, number of florets per spikelet, number of total tillers per plant, number of fertile tillers per plant, lodging index, above-ground biomass per plot, grain yield per plot, harvest index and thousand seed weight data were collected and subjected to analysis using appropriate software.

In addition to phenotypic and genotypic coefficients of variation, the variability of each quantitative trait was evaluated using simple metrics like mean, range, and standard deviation. The formula presented by Singh and Chaudhary (1977) was used to estimate the phenotypic (PCV) and genotypic (GCV) coefficients of variations.

Genotype variance ($\sigma^2 g$) = $\frac{MS_g - MS_e}{r}$

Phenotypic variance ($\sigma^2 p$) = $\sigma^2 g + \sigma^2 e$

Where, $\sigma^2 g$ = genotypic variance MS_g= mean square of genotype MS_e = mean square of error r = number of replications $\sigma^2 e$ = Environmental variance and $\sigma^2 p$ = phenotypic variance.

$$PCV = \frac{\sqrt{phenotypic\ \text{var}iance}}{population\ mean\ for\ the\ trait} x100$$

$$GCV = \frac{\sqrt{genotypic\ \text{var}iance}}{population\ mean\ for\ thet\ rait} x100$$

Heritability (H^2) in broad sense for all characters was computed using the formula adopted by Allard (1960).

$$H^2 = \left[\sigma^2{}_g \Big/ \sigma^2{}_p \right] x100$$

Where; H^2 = heritability in broad sense

Table 1. List and description of tef genotypes used for the study.

No.	Pedigree (Genotype)	No.	Pedigree (Genotype)
1	DZ- Cr-387xRosea (RIL 162)	26	DZ-01-1681 x Alba (RIL 147)
2	DZ- Cr-387xRosea (RIL 14)	27	DZ-01-1681x Alba (RIL 142)
3	DZ- Cr-387xRosea (RIL 106)	28	DZ-01-1681x Alba (RIL 144)
4	DZ- Cr-387xRosea (RIL 196)	29	DZ-01-1681 x Alba (RIL 31)
5	DZ- Cr-387xRosea (RIL 173)	30	DZ-01-1681 x Alba (RIL 87)
6	DZ- Cr-387xRosea (RIL 6)	31	DZ-01-1681 x Alba (RIL 175)
7	DZ- Cr-387xRosea (RIL 132)	32	DZ-01-1681 x Alba (RIL 103)
8	DZ- Cr-387xRosea (RIL 92)	33	DZ-01-1681 x Alba (RIL 76)
9	DZ- Cr-387xRosea (RIL 96)	34	DZ-01-1681 x Alba (RIL 121)
10	DZ- Cr-387xRosea (RIL 117)	35	DZ-01-1681 x Alba (RIL 32)
11	DZ- Cr-387xRosea (RIL 138)	36	DZ-01-1681 x Alba (RIL 78)
12	DZ- Cr-387xRosea (RIL 163)	37	DZ-01-1681 x Alba (RIL 47)
13	DZ- Cr-87xRosea (RIL 7)	38	DZ-01-1681 x Alba (RIL 70)
14	DZ- Cr-387xRosea (RIL 58)	39	DZ-01-1681 x Alba (RIL 97)
15	DZ- Cr-387xRosea (RIL 107)	40	DZ-01-1681 x Alba (RIL 116)
16	DZ- Cr-387xRosea (RIL 53)	41	DZ-01-1681 x Alba (RIL 46)
17	DZ- Cr-387xRosea (RIL 122)	42	DZ-01-1681 x Alba (RIL 30)
18	DZ- Cr-387xRosea (RIL 119)	43	DZ-01-1681 x Alba (RIL 15)
19	DZ- Cr-387xRosea (RIL 1)	44	DZ-01-1681 x Alba (RIL 100)
20	DZ- Cr-387xRosea (RIL 98)	45	DZ-01-1681 x Alba (RIL 134)
21	DZ- Cr-387xRosea (RIL 157)	46	DZ-01-1681 x Alba (RIL 185)
22	DZ- Cr-387xRosea (RIL 155)	47	DZ-01-1681 x Alba (RIL 2)
23	DZ- Cr-387xRosea (RIL 166)	48	DZ-01-1681 x Alba (RIL 48)
24	DZ-Cr-387xRosea (RIL 91)	49	Dagim (DZ-Cr-438 RIL91)
25	DZ-01-1681 x Alba (RIL 120)		

DZ- Debre Zeit, Cr- Cross, Rosea and Alba- Tef cultivars.

The heritability estimates were categorized as low (0-30%), moderate (30- 60%) and high (60% and above) as suggested by Robinson et al. (1949).

Genetic advance under selection (GA) for each character was computed using the formula adopted by Johnson et al. (1955).

$GA = (k)(SDp)(H^2)$, and GA (as % of the mean) = $\left[\frac{(GA)}{\bar{x}}\right] x100$

Where; k = selection differential (with a value of 2.06 at 5% selection intensity), SDp= phenotypic standard deviation, H^2 = heritability in broad sense, $\bar{x}$ = Grand mean. Genetic advance as a percentage mean was categorized as low (0-10%), moderate (10-20% and high (≥20%) as suggested by Johnson et al. (1955).

RESULTS AND DISCUSSION

Analysis of variance

Tests were conducted to check the homogeneity of error variances prior to doing the combined analysis of variance over locations, and all of the traits showed homogeneity of error variances. Consequently, the data were pooled across locations and analyzed, and the results of the combined analysis of variance across the two test locations are presented in Table 2. The mean squares from the pooled analysis of variance over the two locations showed a highly significant location (P≤0.01) effect for almost all traits except for peduncle length and thousand seed weight. The mean squares from the pooled analysis of variance over the two locations also showed highly significant (P≤ 0.01) effects of genotypes for all traits except for number of fertile tillers per plant and thousand seed weight (Table 2). Similar significant results were reported for most traits in earlier studies (Solomon et al*.,* 2009; Jifar et al*.,* 2015; 2017; Tsion, 2016).

The mean squares resulting from the genotype x location interaction were statistically significant (P 0.05) for the number of florets per spikelet and the total number of tillers per plant, but highly significant (P 0.01) for the grain filling period, number of spikelets per panicle, lodging index, above ground biomass, and harvest index. Days to heading, panicle length, culm length, peduncle length, number of primary panicle branches per main shoot, number of florets per spikelet, number of fertile tillers per plant, harvest index, and thousand seed weight

Table 2. Mean squares from the combined analysis of variance of 17 traits of 49 tef genotypes tested at two locations during the 2021/22 main season.

Trait	MSl	MSr(l)	MSg	MSgl	MSE	CV (%)	R^2
	(df=1)	(df=2)	(df=48	(df=48)	(df=72)		
DTH	408.62**	8.49**	15.44**	0.52ns	0.72	1.63	0.97
DTM	519.19**	15.07*	41.96**	6.22ns	4.19	1.84	0.92
GFP	358.29**	1.66ns	25.41**	15.75**	5.75	4.06	0.84
PH	5247.14**	601.60**	129.52**	28.68ns	22.69	4.65	0.91
PL	659.02**	136.56**	42.12**	4.88ns	4.56	5.17	0.92
CL	2187.03**	248.17**	69.38**	21.55ns	20.25	7.36	0.85
PDL	14.28ns	22.20**	8.84**	3.80ns	3.81	9.45	0.75
NSPP	73247.56**	6669.41**	31633.28**	6495.23**	1153.71	5.43	0.97
NPBPMS	393.43**	56.14**	32.44**	3.22ns	4.57	10.18	0.90
NFPS	3.30**	7.17**	1.08**	0.56*	0.33	10.00	0.83
NTTPP	27.19**	16.50**	4.78**	4.14*	2.39	12.09	0.77
NFTPP	95.93**	23.50**	4.28ns	3.82ns	2.99	16.04	0.74
LI	7044.01**	178.13**	437.39**	149.03**	6.44	3.78	0.99
BY	2212656.25**	2389595.03**	1685890.73**	1994820.90**	57757.2	1.78	0.98
GY	1251753.40**	23494.58**	110401.01**	125128.47**	3077.62	2.12	0.99
HI	41.04**	1.93**	4.4089**	3.83**	0.22	2.44	0.97
TSW	0.0015ns	0.018**	0.0017ns	0.0014ns	0.0014	12.68	0.70

*,** Significant at $p \leq 0.05$, and $p \leq 0.01$ probability level respectively and ns= non-significant, Figures in parenthesis indicate degrees of freedom, MSl= Mean Squares of locations, MSr (l) =mean squares of block (location), MSg= Mean squares of genotypes, MSgl = Mean square of genotype x location interaction, MSE = Mean squares of error, CV = Coefficient of variation, R^2 = coefficient of determination. DTH =days to heading, DTM = days to physiological maturity, GFP = grain filling period, PH= Plant height, PL=panicle length, CL= culm length, PDL= peduncle length, NSPP=number of spikelets per panicle, NPPBMS = number of primary panicle branches per main shoot, NFPS =number of florets per spikelet, NTTPP= number of total tillers per plant, NFTPP= number of fertile tillers per plant, LI= lodging index, BY=biomass yield, GY= grain yield, HI = harvest index and TSW= thousand-seed weight.

did not show statistically significant genotype x location interaction (Table 2).

The findings of the current study agree with those of Kebede et al. (2019) which found no significant genotype x location interaction for days to heading, panicle length, culm length, peduncle length, number of florets per spikelet, total number of tillers per plant and thousand seed weight. Similar results were found by Solomon et al. (2009) and Assefa et al. (1999) regarding the genotype x location interaction effect on panicle length. The significant differences observed among the genotypes for grain yield and yield-related traits suggest the presence of substantial variation in the inherent genetic potential of the advanced lines/genotypes studied depicting the possibility of selecting high yielding tef genotypes.

Phenotypic and genotypic coefficient of variation

Genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) are used to measure the variability that exists in a given population. High genotypic coefficients of variation indicate availability of high genetic variation. The GCV ranged from 2.96% for days to physiological maturity to 15.82% for number of primary panicle branches per main shoot, whereas the PCV ranged from 3.62% for days to physiological maturity to 18.42% for number of primary panicle branches per main shoot (Table 3). Sivasubramaniah and Menon (1973) suggested that the values of PCV and GCV can be categorized as low (0-10), moderate (10-20) and high (> 20).

According to this classification the estimates of PCV were moderate for peduncle length (11.18%), number of spikelets per panicle (18.29%), number primary panicle branches per main shoot (18.42%), number of florets per spikelet (13.84%), number of fertile tillers per plant (11.18%), lodging index (17.79%), grain yield (12.27%), harvest index (13.59%) and thousand seed weight (15.17) (Table 3). Previous findings by Solomon *et al*. (2009), Solomon (2010), Ayalew et al. (2011), Habte et al. (2015), Nigus et al. (2016) and Tsion (2016) were also similar to the present results for estimates of phenotypic coefficients of variation for most the tef traits.

On the other hand, GCV were relatively moderate for number of primary panicle branches per main shoot (15.82%), number of spikelets per panicle (14.73%) and lodging index (13.33%). In contrast to the present

Table 3. Estimates of variance components, phenotypic and genotypic coefficients variance, broad sense heritability and expected genetic advance for 17 traits of 49 tef genotypes based on analysis of variance over two test locations.

Traits	Range	Mean ±SE	σ^2g	σ^2p	GCV (%)	PCV (%)	H^2 (%)	GA	GAM (%)
DTH	48-57.50	52.17 ± 0.58	5.32	6.00	4.42	4.70	88.67	4.48	8.59
DTM	105.25-117.50	111.43 ±1.39	10.93	16.24	2.96	3.62	67.31	5.60	5.02
GFP	50.50-64.75	59.04 ±1.66	3.07	13.95	2.97	6.33	22.03	1.70	2.88
PH	91.05-116.80	102.48 ±3.33	31.61	58.29	5.49	7.45	54.24	8.54	8.34
PL	34.30-48.95	41.31 ±1.47	11.44	16.21	8.19	9.75	70.56	5.86	14.19
CL	51.40-69.70	61.17 ±3.09	13.49	35.19	6.00	9.70	38.33	4.69	7.67
PDL	16.30-26.05	20.70 ±1.4	1.60	5.36	6.11	11.18	29.88	1.43	6.89
NSPP	400.33-816.17	625.33 ±23.97	8485.70	13080.00	14.73	18.29	64.88	153.07	24.48
NPPBPMS	16.17-28.42	20.99 ±1.48	11.03	14.95	15.82	18.42	73.78	5.88	28.03
NFPS	4.55-7.03	5.76 ±0.46	0.21	0.64	7.90	13.84	32.59	0.54	9.30
NTTPP	10.05-14.95	12.79 ±1.14	0.16	1.11	3.12	8.26	14.26	0.31	2.43
NFTPP	8.85-13.30	10.77 ±1.25	0.25	1.45	4.66	11.18	17.42	0.43	4.02
LI	38.50-88.25	67.21 ±1.94	80.25	142.88	13.33	17.79	56.17	13.85	20.61
BY	10750-14750	13415.18±548.53	586283.90	1593897.86	5.69	9.38	37.00	956.63	7.10
GY	2222.20-2904.7	2615.49 ±38.92	46017.32	103030.91	8.20	12.27	45.00	295.33	11.29
HI	16.78-37.57	19.78 ±3.26	3.53	7.22	9.49	13.59	48.80	2.71	13.68
TSW	0.24-0.34	0.29 ±0.03	0.001	0.002	8.26	15.17	29.64	0.03	9.28

DTH =days to heading, DTM = days to physiological maturity, GFP = grain filling period, PH= Plant height, PL=panicle length, CL= culm length, PDL= peduncle length, NSPP=number of spikelets per panicle, NPPBPMS = number of primary panicle branches per main shoot, NFPS =number of florets per spikelet, NTTPP= number of total tillers per plant, NFTPP= number of fertile tillers per plant, LI= lodging index, BY=biomass yield, GY= grain yield, HI = harvest index and TSW= thousand-seed weight, SE=Standard error of mean, σ^2g = Genotypic variance, σ^2p=Phenotypic variance, PCV=phenotypic coefficients of variation, GCV= genotypic coefficients variation, H^2 =heritability in broad sense, GA= genetic advance, GAM= genetic advance as percentage of mean.

findings, Nigus et al. (2016) and Kebede et al. (2019) reported high value of GCV for lodging index and number of spikelet per panicle, respectively. Solomon (2010) and Ayalew et al. (2011) reported low value of GCV for day to maturity, harvest index and grain filling period.

PCV is usually the reflection of the effects of genotypes and environment and if PCV is greater than GCV, it indicates that the environment has a greater influence on the phenotypic expression of the trait than the gene effect (Habte et al., 2015). High GCV values imply greater potential for these traits to be improved through selection. Since improvement efforts typically concentrate on traits with higher values of GCV estimates, GCV provides a better measure of the extent of genetic variation. The number of spikelets per panicle, the number of primary panicle branches per main shoot and lodging index in this study all had moderate GCV values, indicating it opportunity for improvement. As a result, GCV allows for a better assessment of the extent of genetic variation among genotype (Solomon et al., 2013).

Heritability and expected genetic advance

Broad sense heritability values of the different traits based on the combined analyses of variance ranged from 14.26% for number of total tillers per plant to 88.67% for days to heading (Table 3). High heritability estimate was observed for days to heading (88.67%), days to physiological maturity (67.31%), panicle length (70.56%), number of spikelets per panicle (64.88%) and number of primary panicle branches per main shoot (73.78%). In line with the current findings similar results were reported by Solomon (2010) and Habte et al. (2015). Genetic advance as percentage of mean in present study ranged from 2.43% for number of fertile tillers per plant to 28.03 % for number primary panicle branches per main shoot (Table 3). In the present study, the number of primary panicle branches per main shoot (28.03%), number of spikelets per panicle (24.48%) and lodging index (20.61%) recorded high genetic advance as percentage of mean. For most traits' similar findings to that of the present study were also reported by Abel Debebe et al. (2012), Jifar and Gugssa (2013), Jifar et al. (2015, 2017) and Kebede et al. (2019).

The estimate of genetic advance as percentage of mean is more useful as a selection tool when considered jointly with heritability estimates (Johnson et al., 1955). Therefore, a high heritability together with a high genetic advance as a percentage of mean imply the importance of additive genes for the development of the traits, and this might make selection more successful. Both the number of spikelets per panicle and the number of primary panicle branches per main shoot showed high

heritability estimates in the current study along with high genetic advance as a percentage of mean, whereas panicle length showed high heritability values along with moderate genetic advance as a percentage of mean. For grain yield and harvest index, it was observed that there was a moderate heritability value along with a moderate genetic advance as a percentage of the mean. Similar to this, Solomon (2010) also noted a greater heritability value along with genetic advance as a percentage of mean for lodging index and panicle length.

To improve traits of interest, estimates of genotypic and phenotypic coefficients of variation, heritability, and genetic progress as a percentage of mean are crucial (Denton and Nwangburuka, 2011). The high heritability estimates along with low genetic advance as percentage of mean indicate that non-additive type of gene action and genotype x environment interaction play a significant role in the expression of the trait (Fatema et al., 2011). High GCV, heritability and genetic advance as percentage of mean for traits could be an excellent tool for improvement through selection of high performing genotypes (Akbar et al., 2003).

Conclusion

The current study showed a presence of significant genetic variations among tef genotypes for grain yield and yield-related traits, which allows plant breeders to develop improved varieties for the traits of interest and use them in the breeding program. The higher heritability estimate coupled with high genetic advance as percentage of mean found for important agronomic traits in the present study suggest the possibility of improving tef grain yield through direct selection of superior genotypes through phenotypic based selection. Thus, there is an opportunity of exploiting the existing variability in tef improvement programs through selection and hybridization.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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