OPEN ACCESS

African Journal of Agricultural Research

6 December, 2018
ISSN 1991-637X
DOI: 10.5897/AJAR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJAR

The African Journal of Agricultural Research (AJAR) is a double blind peer reviewed journal. AJAR publishes articles in all areas of agriculture such as arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, post-harvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, forestry, freshwater science, horticulture, soil science, weed biology, agricultural economics and agribusiness.

Indexing

Science Citation Index Expanded (ISI), CAB Abstracts, CABI's Global Health Database
Chemical Abstracts (CAS Source Index), Dimensions Database, Google Scholar
Matrix of Information for The Analysis of Journals (MIAR) Microsoft Academic
ResearchGate, The Essential Electronic Agricultural Library (TEEAL)

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Agricultural Research is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF). The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editorial Office: ajar@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJAR

Submit manuscript online http://ms.academicjournals.org

Academic Journals

73023 Victoria Island, Lagos, Nigeria

ICEA Building, 17th Floor, Kenyatta Avenue, Nairobi, Kenya

Editors

Editorial Board Members

Table of Content

Vol. 13(49), pp. 2771-2779, 6 December, 2018
DOI: 10.5897/AJAR2018.13340
Article Number: 43C93BE59475
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Identification of sequence-characterized amplified regions (SCARs) markers linking resistance to powdery mildew in chilli pepper (*Capsicum annuum* L.)

Rajesh R. Wankhade* and Madhukar S. Wadikar

Department of Botany, Vinayakrao Patil College, Vaijapur, District- Aurangabad, Maharashtra, 423 701 - India.

Received 20 June, 2018; Accepted 16 October, 2018

Powdery mildew disease in chilli pepper caused by *Leveillula taurica* has been affecting chilli pepper grown in greenhouse and open fields. Inheritance of powdery mildew disease resistance is complex and least understood. In the present study, we identified SCAR markers using F_2 mapping populations developed from a cross between a resistant parental line 'Odisha Local' and a parental line susceptible to powdery mildew '9907-9611'. The nucleotide sequence obtained from a genome of resistant chilli pepper line 'Odisha Local' using OPA15 primer showed partial identity with RPP13 like disease resistant protein. The molecular markers developed in this study will be very helpful in chilli pepper breeding programs for powdery mildew resistance for indirect selection of the resistant plants.

Key words: Chilli pepper, powdery mildew, SCAR markers.

INTRODUCTION

Chilli pepper (*Capsicum annuum* L.) is an economically important spice crop that is widely cultivated in India as well as in tropical and sub-tropical countries. Its primary use is for culinary purposes, as a spice added to various dishes and sauces. Some varieties are commercially cultivated for capsaicin. India is the largest exporter of chilli as it exported 0.4 million tones of dry chilli in 2016-17 (Spices Board, 2018).

Chilli pepper is susceptible to many fungal and bacterial diseases affecting yield. Among fungal diseases, powdery mildew caused by *Leveillula taurica* (LEV.) is an obligate fungal plant pathogen belonging to the ascomycetes, which infects various vegetable crops, resulting in very significant yield losses and quality deterioration. The incidence of the powdery mildew disease in Chilli pepper has been showing an upward trend in both open field and protected net-houses worldwide (Jinkwan et al., 2017). It is one of the important diseases causing up to 80% loss in yield due to severe defoliation and reduction in photosynthesis resulting in less number of fruits and affecting quality of marketable yield (Mathur et al., 1972; Sivaprakasam et al., 1976; Gohokar and Peshney, 1981). The disease appears as white powdery coating on ventral side of leaves and

*Corresponding author. E-mail: rrwankhade@rediffmail.com.

correspondingly produces yellow patches on dorsal side. It usually spreads to branches of plants and fruits resulting in dropping of affected fruits.

The fungi causing powdery mildew is epiphytic and *L. taurica* is an endophytic fungus, which makes chemical control difficult (Elad et al., 2007). Therefore, developing powdery mildew disease resistance in chilli pepper is one of the main objectives of breeding programs (Jinkwan et al., 2017).

Phenotypic screening by assays is used commonly in breeding programs (Ottoman et al., 2009); however, they are expensive, laborious, inefficient and time consuming. Closely linked molecular markers to resistance genes can help breeders overcome these difficulties. Molecular markers are very effective and efficient mean in plant breeding for indirect selections and introgression of traits in certain genotypes. Thus, the identification of markers linked to genes controlling resistance/tolerance to biotic and abiotic stresses plays an important role in plant breeding programs. Therefore, globally, the main goal of pepper breeders is to develop disease resistant varieties or planting material (Jinkwan et al., 2017).

Random amplified polymorphic DNA (RAPD) technique was developed by Williams et al. in 1990 by using random primers which allows quick construction of genetic maps or the saturation of genomic regions of interest (Paran and Michelmore, 1993). RAPD technique is easy, using less quantity of DNA. However, RAPD has some limitations and its results are not always reproducible; it shows dominant inheritance and cannot be converted to codominant markers (Mishra, 2014), and is sensitive to changes in reaction conditions (Paran and Michelmore, 1993). To remove these limitations, Paran and Michelmore (1993) had developed sequence-characterized amplified regions (SCARs) as PCR based molecular markers. SCAR is a genomic DNA fragment that is identified by PCR amplification using a pair of specific oligonucleotide primers (Paran and Michelmore, 1993). SCAR markers have many advantages over RAPD marker. They are less sensitive to reaction condition (Paran and Michelmore, 1993). PCR amplification of the SCARs is reproducible and easy to score (Weng et al., 1998). Therefore, analysis using SCAR markers are fast, easy and very straightforward. The aim of the present study was to identify SCAR markers linked to powdery mildew gene that would help breeders in indirect selection for the trait in efficient and fast manner.

MATERIALS AND METHODS

Field screening and disease evaluation

In rainy season of 2012, an experiment was conducted to screen 19 chilli pepper genotypes against powdery mildew in field conditions. The observations were recorded at 15 days interval starting from 60 to 180 days after transplanting using 0-9 disease scale (Mayee and Datar, 1986) (Table 1). The per cent disease index (PDI) was calculated as per the formula given by Wheeler (1969):

$$\text{PDI} = \frac{\text{Sum of numerical values grades}}{\text{Number of plants observed}} \times \frac{100}{\text{Maximum disease rating}}$$

Plant materials

Parental lines “Odisha Local” and 9907-9611 highly resistant and susceptible to powdery mildew, respectively were selected for the present experiment. The F_1 plants were derived from a cross between “Odisha Local” and 9907-9611. F_1 hybrid was self-pollinated to produce F_2 seeds in summer of 2013. In rainy season of 2013, we evaluated 199 F_2 plant populations along with parental lines for *L. taurica* disease resistance and observations were taken till 180 days of planting using 0-9 disease scale (Mayee and Datar, 1986) (Table 1). Powdery mildew infection was scored by the appearance of mycelia growth on the leaf surface of plants and susceptible parental line also used as susceptible check (Figure 4). There was no fungal hyphae growth on powdery mildew resistant “Odisha Local” (Figure 1). In F_2 population, plants scoring zero were considered as immune, plants scored as one to seven were phenotyped as highly resistant to moderately susceptible in various classes and plant scored nine were phenotyped as susceptible. Jinkwan et al. (2017) also adapted such method to score powdery mildew disease pressure in pepper.

In SCAR marker development procedure, unequal DNA quantity from individual plants increases variation in PCR and electrophoresis quantification of alleles. Daniels et al. (1998) suggested that the DNA pooling method reduces variation, is efficient, fast reliable method to detect differences in allele frequencies and to handle large numbers of sample. To achieve this, 20 plants each were selected in highly resistant “Odisha Local” parent and highly susceptible “9907-9611” based on phenotypic scores relating to disease reaction for making two separate DNA pools, that is, resistant and susceptible DNA pool. Young, fresh and healthy leaves were collected from selected plants of both the parental lines and stored with silica gel in separate zip-lock plastic bags.

Genomic DNA extraction

Genomic DNA from susceptible and resistant parental plant tissue was extracted separately to make resistant and susceptible DNA pool, following procedure by Wang et al. (2011) and based on guanidinium thiocyanate reagent. The quality and quantity of the gDNA were analyzed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The pooled samples were used to identify putative markers for the powdery mildew resistance in Chilli.

RAPD

PCR was performed as per RAPD method described by Williams et al. (1990). The DNA pool from susceptible and resistant plant population and 200 different decamer oligonucleotide primers (Series A, B, C, D, E, F, G, K, L and BA from Operon Technologies Inc., Alameda, CA, USA) were used in PCR. The series represents different primer kits of RAPD markers. Each kit contains 20 individual 10-mer primers (supplied at a minimum quantity of 50 nmole per primer). In the RAPD technique, a single 10mer of arbitrary sequence is used as a primer in PCR to amplify genomic DNA where the sequence of the DNA is completely unknown. The reaction mixtures contained 1 × PCR Buffer (10 mM Tris pH 8.8, 50 mM KCl, 0.08% Nonidet P40; Fermentas, Lithuania), 160 µM of each dNTP, 530 pM oligonucleotide primer, 1.5 mM $MgCl_2$, 35 ng of

Table 1. Scale for Powdery mildew disease in chilli.

Grade	Symptoms and host reaction
0	Immune (I) - No symptom of powdery mildew
1	Highly Resistant (HR) - Small scattered powdery mildew specks covering 1% or less leaf area
3	Resistant (R) - Small powdery lesions covering 1-10% of leaf area
5	Moderately Resistant (MR) - Powdery lesions enlarged covering 11-25% of leaf area
7	Moderately Susceptible (MS) - Powdery lesions coalesce to form big patches covering 26-50% of leaf area
9	Highly Susceptible (HS) - Big powdery patches covering 51% or more of leaf area and defoliation occur

Figure 1. 'Odisha Local' (Powdery milder resistant) and 9907-9611 (Powdery mildew sensitive).

template DNA, 0.5 U Taq DNA Polymerase (Fermentas, Lithuania) in a final reaction mixture of 15 µl. Amplification was carried out in Biometra T1 thermal cycler programmed for 94°C for five min, followed by 39 cycles at 94°C for 1 min, 42°C for 1 min and 72°C for 1 min 30 s, terminating with a final extension at 72°C for 10 min. Amplification products were separated on 1.5% agarose gels containing 0.1% EtBr. Fragments were visualised under a UV transilluminator and archived using DigiGenius (Syn-Gene) system. Those products that were able to differentiate the studied DNA pools were isolated from agarose gel using MiniElute (Qiagen). The amplified products were cloned using TOPO TA Cloning Kit (Thermofisher) following the manufacturer's instructions and sequenced using BigDye terminator cycle sequencing kit (Applied Biosystems) using an automated DNA sequencing system (3130 Genetic Analyzer -Applied Biosystems) (Sambrook et al., 1989). Two independent clones for each set were sequenced in both orientations by using universal M13 forward and reverse primers. Nucleotide sequences were analyzed using the Contig-Express and AlignX tools available in Vector NTI software version 6.0 (Invitrogen) (Jinkwan et al., 2017; Kunkalikar et al., 2012).

Each RAPD primer was tested at least three times to ensure reproducibility of polymorphism and the banding patterns. The OPA-15 primer (Table 2) consistently yielded 1.1 Kb and 0.9 Kb amplicons in susceptible and resistant parents respectively. The amplified products were sequenced and all the obtained sequences submitted to the GenBank database (MH172153). Nucleotide sequences were analyzed using the Contig-Express and AlignX tools available in Vector NTI software version 6.0 (Invitrogen) and then compared with corresponding sequences available in GenBank (Kunkalikar et al., 2012).

Development of SCAR markers

On the basis of alignment of sequences of 1.1 Kb and 0.9 Kb DNA fragments amplified with OPA15 primers, the specific forward

Table 2. RAPD primers amplifying DNA fragments specific for resistant and susceptible pools in Odisha Local" x 9907-9611 population.

Primer	Sequence
SR1	GGTGCGGGAA
SR2	GTTTCGCTCC
OPV19	GGGTGTGCAG
OPA15	TTCCGAACCC
OPY 02	CATCGCCGCA
OPC02	GTGAGGCGTC
OPA 17	GACCGCTTGT
OPM 07	CCGTGACTC

Table 3. Primers for SCAR markers.

Primer	Sequence
OPA15-SFP	CGAATAAGGGCTTTGGCCTAATTCA
OPA15-RFP	GATTTAGTCGAGGTGCATGAAAGT
OPA15-CRP	TAYSARGCAGARYTASWRWTCCAAGT

Table 4. List of IUPAC degenerate nucleotide codes.

Codes	Nucleotide	Codes
A	Adenine	A
C	Cytosine	C
G	Guanine	G
T	Thymine (DNA)	T
U	Uracil (RNA)	U
W	Weak	A/T
S	Strong	C/G
M	Amino	A/C
K	Keto	G/T
R	Purine	A/G
Y	Pyrimidine	C/T
B	Not A	C/G/T
D	Not C	A/G/T
H	Not G	A/C/T
V	Not T	A/C/G
N	Any	A/C/G/T

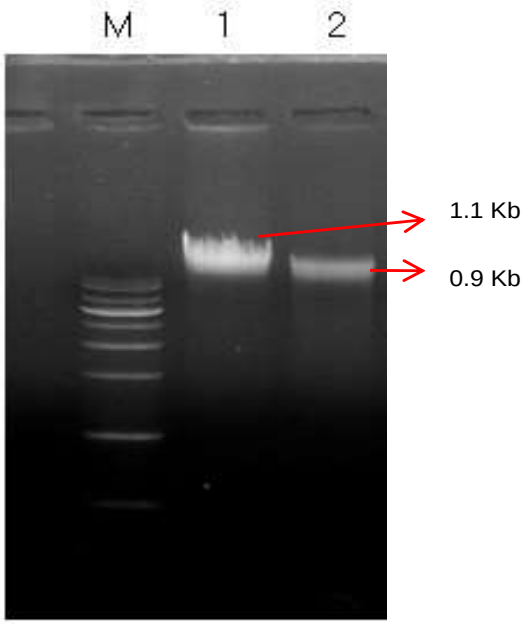

Figure 2. Amplicons of 1.1 Kb (susceptible phenotype) and 0.9 Kb (resistant phenotype)

primers OPA15SFP and OPA15-RFP specific to susceptible and resistant plants respectively along with a common reverse primer OPA-CRP (Tables 3 and 4) were designed to develop SCAR markers. These primers were used in PCR amplification of genomic DNA of "Odisha Local" and 9907-9611.

Screening F2 population using SCAR marker

Polymorphism in OPA15 SCAR markers was examined in 199 F_2 plants obtained by crossing resistant and susceptible parental lines, "Odisha Local" and 9907-9611. The genomic DNA of plants was used to obtain amplicons in primers OPA15SFP, OPA15-RFP and OPA-CRP. The plants were scored based on size of amplicons: The plants with amplicons of 0.9 Kb showing resistant phenotype were graded as "1", and with 1.1 Kb amplicon showing susceptible phenotype were graded "3". The plants showing both 0.9 Kb and 1.1 Kb amplicons were graded "2" (Figure 2). The genotypic and

phenotypic scores were analysed for chi-square test and correlation.

RESULTS

RAPD analysis

In RAPD analysis of plants in F_2 population with 200 decamer oligonucleotide primers, eight primers showed polymorphism. The OPA-15 primer was found consistent in yielding 1.1 Kb and 0.9 Kb amplicons in susceptible and resistant parents respectively.

SCAR markers

The PCR amplification of genomic DNA of "Odisha Local" and 9907-9611 using OPA15 primers yielded amplicons of 0.9 Kb and 1.1 Kb respectively (Figure 2).

The polymorphism of SCAR markers was studied in 199 F_2 plants obtained by crossing same resistant and susceptible parental lines, "Odisha Local" and 9907-9611. Out of 199 F_2 plants, 14 plants were homozygous resistant, 171 plants were segregating for the trait and 14 plants were homozygous susceptible (Figure 3).

A Chi-square (χ^2) test for goodness-of-fit (Table 6) was tested with the hypothesis of marker score aligned with phenotypic scores of the F2 population of 199 individual plants (Table 7a and b). The hypothesis was considered appropriate for a probability (P) value between 0.75 and 0.50 (Table 6). Association analysis between genotypic and phenotypic scores of F2 population was carried out. The correlation of marker scores with phenotypic scores were highly significant (r=0.623).

The nucleotide sequence obtained from a genome of resistant chilli pepper line 'Odisha Local' using SCAR-OPA15 primer showed partial identity with RPP13-like disease resistant protein (GenBank Accessions XM_016717781, XM_016717782, XM_016717784).

DISCUSSION

Identification of resistance source for powdery mildew disease caused by *L. taurica* is important in resistance breeding. Marker assisted selection is one of the most widely used applications in breeding programs (Foolad, 2007). The process reduces breeding time and allows stacking of desirable genes in an otherwise elite line. Therefore, development of molecular markers closely linked to the gene of interest for powdery mildew resistance is of high importance for breeders.

We worked to generate linked markers for molecular breeding programme. To identify resistant germplasm source of chilli pepper, a total of 19 genotypes including three commercial hybrids, eight commercial varieties, seven local collections and a susceptible check Byadgi Kaddi were screened for powdery mildew resistance in epiphytotic conditions. Lines "Odisha Local" and 9907-

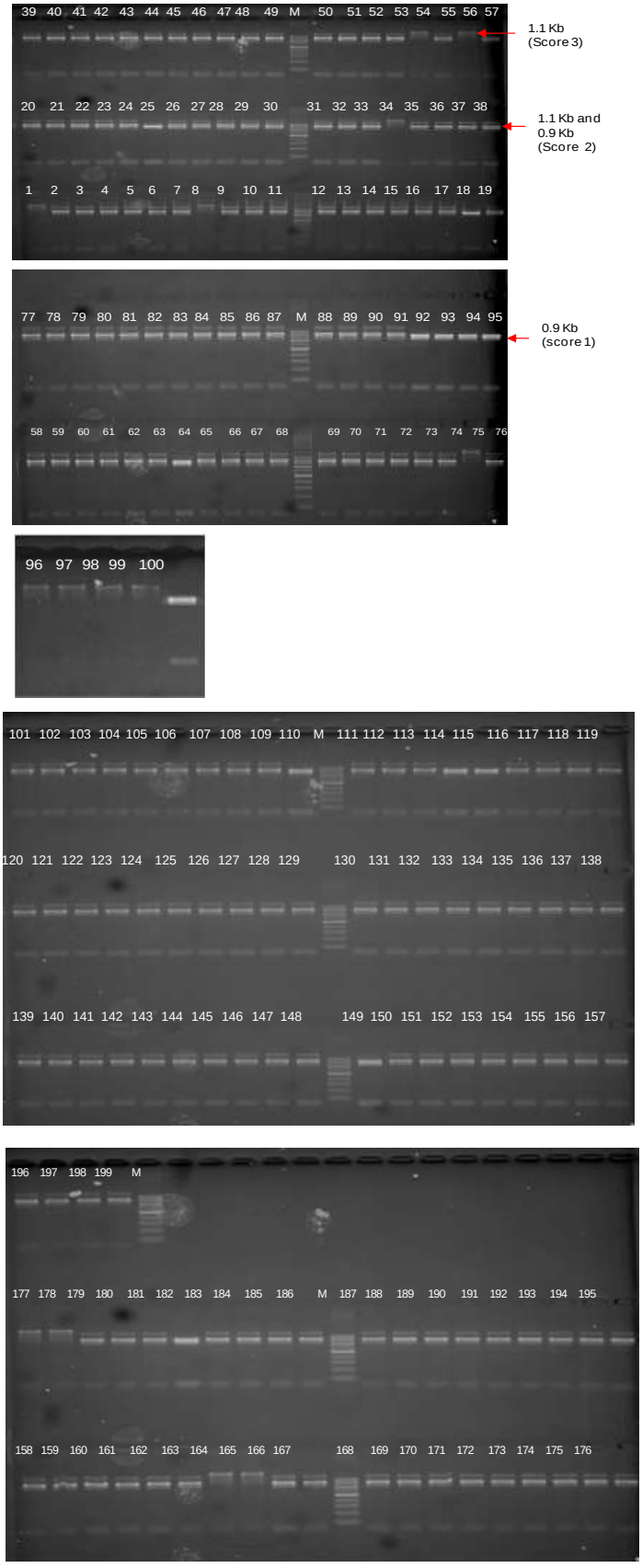

Figure 3. PCR products amplified by the SCAR primers OPA15-SFP, OPA15-RFP and OPA15-CRP. M, GeneRuler 1000 bp.

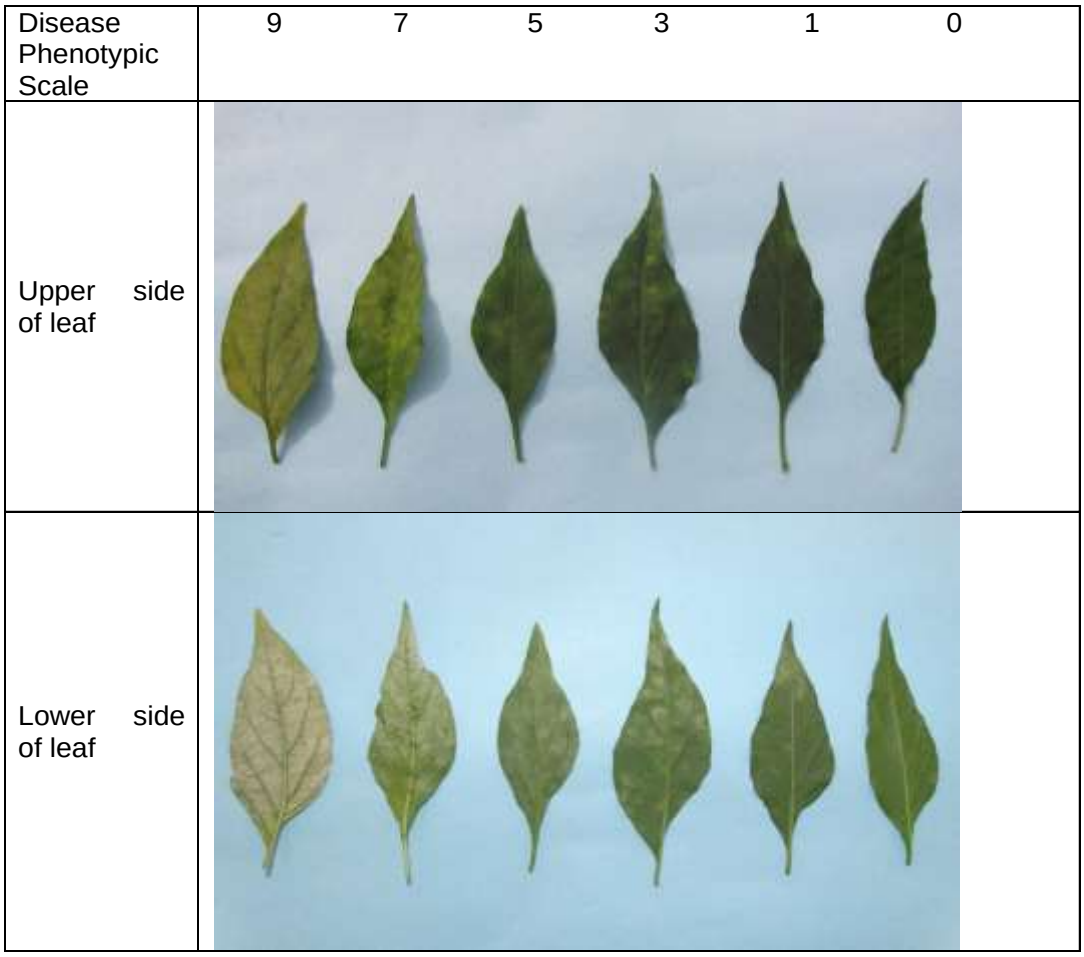

Figure 4. Powdery mildew symptoms on upper and lower leaves of Chilli plant and corresponding phenotypic scores.

9611 resistant and susceptible to powdery mildew, respectively were identified. Resistant and susceptible DNA pools from these two lines were used to further develop SCAR markers for MAS. The polymorphism of SCAR-OPA15 was also confirmed on F_2 population derived from crossing lines "Odisha Local" and 9907-9611. This molecular tool in the hands of breeders helps in indirect selection of genotypes saving time and resources in field screening.

The powdery mildew resistant 'Odisha Local' genome sequence obtained by SCAR-OPA 15 primer shows identity with allele RPP13-like protein. Bittner-Eddy et al. (2000) suggested that the RPP13 locus in Arabidopsis accession Nd-1, contains either a single gene capable of multiple isolate recognition or a group of tightly linked genes that is responsible for resistance against *P. parasitica* isolate Maks9 with localized necrotic flecks on host plant tissue and no pathogen reproduction. RPP13-like allele is implicated in conferring resistance to biotrophic fungal pathogens. The RPP13 resistance protein guards the plant against pathogens that contain an appropriate avirulent protein because of an indirect interaction with this avirulent protein. That triggers a defense system including the hypersensitive response, which restricts the pathogen growth. In contrast to other resistance proteins, RPP13-like protein works independently of ESD1 and NSD1 proteins and does not require the accumulation of salicylic acid, suggesting the existence of an independent signaling pathway (Bittner et al., 2000). ESD1 protein causes early flowering independently of photoperiod, moderate increase of hypocotyl length, shortened inflorescence internodes, and altered leaf and flower development. Also, the NSD1 gene provides instructions for making a protein that functions as a histone methyltransferase. Histone methyltransferases are enzymes that modify structural proteins called histones, which attach (bind) to DNA and give chromosomes their shape. Murthy and Deshpande (1997) reported that the genes in two different powdery mildew resistant parents in chilli pepper showed allelic differences in controlling resistance for powdery mildew at least at few loci. Shifriss et al. (1992)

Table 6. Genotypic and phenotypic scores in the F2 population.

Marker data	Powdery mildew disease grade	1	2	3	Chi Square test (P value)
	Expected genotype	**Homozygous resistant**	**Heterozygous**	**Homozygous susceptible**	-
Phenotypic data	PM Scores	0	1- 7	9	-
	Disease reaction	Immune	Highly Resistant to moderately susceptible	Highly susceptible	-
	Number of plants in phenotypic class	14	171	14	-
Genotypic data	Number of plants in genotypic class	13 (Numbers of plants with genotype *aa*, SCAR-OPA15)	172 (Number of plants with genotype *ab*, SCAR-OPA15)	14 (Numbers of plants with genotype *bb*, SCAR-OPA15)	0.0827 (0.75 -0.50)

Table 7a. Comparison of powdery mildew phenotypic and marker (genotype) scores on F_2 generation individual plants of the cross "Odisha Local x 9907-9611".

F2 Plant number	Phenotypic Score	Marker Score	F2 Plant number	Phenotypic Score	Marker Score	F2 Plant number	Phenotypic Score	Marker Score	F2 Plant number	Phenotypic Score	Marker Score
1	9	3	26	1	2	51	3	2	76	5	2
2	7	2	27	1	2	52	5	2	77	5	2
3	5	2	28	3	2	53	5	2	78	5	2
4	3	2	29	3	2	54	9	3	79	5	2
5	3	2	30	1	2	55	5	2	80	7	2
6	1	2	31	1	2	56	9	3	81	7	2
7	3	2	32	7	2	57	3	2	82	7	2
8	9	3	33	1	2	58	7	2	83	5	2
9	3	2	34	9	3	59	7	2	84	7	2
10	7	2	35	3	2	60	3	2	85	7	2
11	7	2	36	1	2	61	1	2	86	5	2
12	7	2	37	1	2	62	1	2	87	5	2
13	7	2	38	7	2	63	1	2	88	5	2
14	7	2	39	5	2	64	0	1	89	7	2
15	7	2	40	5	2	65	1	2	90	7	2
16	0	2	41	1	2	66	1	2	91	7	2
17	3	2	42	3	2	67	5	2	92	0	1
18	0	1	43	3	2	68	5	2	93	0	1
19	1	2	44	3	2	69	5	2	94	0	1
20	1	2	45	7	2	70	1	2	95	0	1
21	3	2	46	3	2	71	1	2	96	9	3
22	3	2	47	5	2	72	5	2	97	9	3
23	1	2	48	3	2	73	5	2	98	9	3
24	3	2	49	5	2	74	7	2	99	9	3
25	0	1	50	1	2	75	9	3	100	0	1

showed that, the disease resistance expression in doubled-haploid variety HV-12 was due to the restriction in pathogen infection, its colonization and leaf defoliation. The inheritance of resistance to powdery mildew in *C. annuum* involves several loci, which demand stronger selection in generations to get homozygosity (Blat et al., 2005). The mode of inheritance for powdery mildew resistance in chilli pepper is complex and inheritance study has also indicated dominant type of resistance to powdery mildew (Anand et al., 1987). This type of durable polygenic resistance is more difficult to be overcome by pathogenic strains (Van der Plank, 1968).

Table 7b. Comparison of powdery mildew phenotypic and marker (genotype) scores on F_2 generation individual plants of the cross "Odisha Local x 9907-9611".

F2 Plant number	Phenotypic Score	Marker Score	F2 Plant number	Phenotypic Score	Marker Score	F2 Plant number	Phenotypic Score	Marker Score	F2 Plant number	Phenotypic Score	Marker Score
101	3	2	126	1	2	151	3	2	176	7	2
102	1	2	127	7	2	152	3	2	177	9	3
103	1	2	128	5	2	153	3	2	178	9	3
104	3	2	129	5	2	154	7	2	179	3	2
105	1	2	130	3	2	155	7	2	180	3	2
106	1	2	131	3	2	156	3	2	181	7	2
107	1	2	132	1	2	157	3	2	182	0	1
108	1	2	133	3	2	158	1	2	183	5	2
109	1	2	134	5	2	159	3	2	184	3	2
110	0	1	135	7	2	160	1	2	185	3	2
111	1	2	136	3	2	161	7	2	186	1	2
112	1	2	137	5	2	162	3	2	187	1	2
113	1	2	138	5	2	163	5	2	188	3	2
114	0	1	139	3	2	164	9	3	189	7	2
115	0	1	140	1	2	165	9	3	190	7	2
116	3	2	141	1	2	166	3	2	191	7	2
117	7	2	142	1	2	167	3	2	192	7	2
118	3	2	143	1	2	168	3	2	193	5	2
119	5	2	144	1	2	169	3	2	194	3	2
120	5	2	145	7	2	170	5	2	195	3	2
121	1	2	146	1	2	171	1	2	196	3	2
122	5	2	147	1	2	172	7	2	197	3	2
123	3	2	148	1	2	173	7	2	198	1	2
124	1	2	149	0	1	174	5	2	199	1	2
125	3	2	150	1	2	175	3	2			

Jinkwan et al. (2017) investigated a powdery mildew disease inheritance in two F2 populations VK515 and PM Singang. The authors revealed that the single dominant locus PMR1 is responsible for inheritance of powdery mildew. One SCAR and five SNP molecular markers were identified in PMR1 locus.

The high heritability of disease resistance shows that the powdery mildew infection reaction in general is not so much influenced by environmental conditions. It was also observed by Blat et al. (2005).

The genome of chilli pepper is large and complex with high genetic variability. The SCAR-OPA15 markers developed in this study could be tested to identify breeding lines with different genetic background for resistance to powdery mildew. Our findings contribute to continuous improvement and generation of new chilli pepper hybrids.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank chilli growers and farmers for allowing access to their fields and providing information on prevalence of powdery mildew diseases in chilli pepper crop. Authors also appreciate Suresh Kunkalikar, Mahyco Research Center, Jalna, Maharashtra, India for critical review of this manuscript.

REFERENCES

Anand N, Deshpande AA, Sridhar TS (1987). Resistance to powdery mildew in an accession of *Capsicum frutescens* and its inheritance pattern. Capsicum Eggplant Newsletter 6:77-78.

Blat SF, Costa CP, Vencovsky R, Sala FC (2005). Inheritance of resistance to *Leveillula taurica* (LEV.) ARN. IN *Capsicum annuum* L. Science Agriculture 62:40-44.

Bittner-Eddy PD, Crute IR, Holub EB, Beynon L, Plant J (2000). *RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. The Plant Journal 21:177-188.

Daniels D, Holmans P, Williams N, Turic D, McGuffin P, Plomin R, Michael J, Owen MJ (1998). A Simple Method for Analyzing Microsatellite Allele Image Patterns Generated from DNA Pools and Its Application to Allelic Association Studies. American Journal of

Human Genetics 62:1189-1197.
Elad Y, Messika Y, Brand M, David DR, Sztejnberg A (2007). Effect of microclimate on *Leveillula taurica* powdery mildew of sweet pepper. Phytopathology 97:813-824.
Foolad MR (2007). Genome mapping and molecular breeding of tomato. International Journal of Plant Genomics doi:10.1155/2007/64358.
Gohokar RT, Peshney NI (1981).Chemical control of powdery mildew of chilli. Indian Journal of Agricultural Science 51:663-665.
Jinkwan J, Jelli V, Koeun H, Hea-Young L, Gyung JC, Hee JL, Doil C, Byoung-Cheorl K (2017). Molecular Mapping of PMR1, a Novel Locus Conferring Resistance to Powdery Mildew in Pepper (*Capsicum annuum*)", Frontiers in Plant Science 8:2090.
Kunkalikar SR, Sudarsana P, Bhanupriya MA, Prem AR, Tsung-Chi C, Shyi-Dong Y, Rayapati A. Naidu, Usha BZ, Kankanallu SR (2011). "Importance and Genetic Diversity of Vegetable-Infecting Tospoviruses in India", Phytopathology 101(3):367-376.
Mathur RI, Singh G, Gupta RBL (1972). Chemical control of chilli (*Capsicum annuum*) caused by *Leveillula taurica*. Indian Journal of Mycology and Plant Pathology 2:182-183.
Mishra KK, Fougat RS, Ballani A, Thakur V, Jha Y, Bora M (2014). Potential and application of molecular markers techniques for plant genome analysis. International Journal of Pure and Applied Bioscience 2(1):169-188.
Murthy HMK, Deshpande AA (1997). Studies on genetics of powdery mildew (*Leveillula taurica* (Lév.) Arn.) resistance in chilli (*Capsicum annuum* L.). Vegetable Science 24:127-131.
Ottoman RJ, Hane DC, Brown CR, Yilma S, James SR, Mosley AR, Crosslin JM, Vales MI (2009). Validation and implementation of marker-assisted selection (MAS) for PVY resistance (*Ryadg* gene) in a tetraploid potato breeding program. American Journal of Potato Research 86:304-314.
Paran I, Michelmore RW (1993). Development of reliable PCR-based markers linked tp downey mildew resistance genes in lettuce. Theor Appl Gene 85:985-993.
Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Sivaprakasam K, Jaganathan R, Pillayarsamy K, Anavaradham (1976). Control of powdery mildew of chillies. The Madras Agricultural Journal 63:52-54.
Van Der Plank JE (1968). Disease resistance in plants. New York: Academic Press P 206.
Wang TY, Wang L, Zhang JH, Dong WH (2011). A simplified universal genomic DNA extraction protocol suitable for PCR. Genetics and Molecular Research 10:519-525.
Weng C, Kubisiak TL, Stine M (1998). SCAR markers in a longleaf pine x splash pine family. Forest Genetics 5(4):239-247.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.
Spices Board (2018). Itemwise export of spices from India. Webpage: Spices Board http://www.indianspices.com/sites/default/files/Major%20Item%20wise%20Export%202017-18.pdf

Vol. 13(49), pp. 2780-2787, 6 December, 2018
DOI: 10.5897/AJAR2018.13481
Article Number: 5720C6259490
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic variability in agronomic traits and associations in sorghum [(*Sorghum bicolor* (L.) Moench)] genotypes at intermediate agro-ecology sorghum growing areas of Ethiopia

Adane Gebreyohannes[1*], Taye Tadesse[1], Amare Seyoum[1], Habte Nida[1], Amare Nega[1], Tsegau Senbetay[2] and Chalachew Endalemaw[1]

[1]Ethiopian Institute of Agricultural Research, Melkassa Agricultural Research Center, Adama, Ethiopia.
[2]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia.

Received 22 August, 2018; Accepted 8 November, 2018

The present study consists of 14 sorghum genotypes evaluated at Bako, Jimma and Mechara research centers to study genetic variability and interrelationships of traits with grain yield. The experiment was conducted by using randomized complete block design (RCBD) with three replications during 2014 and 2015 main rainy season. Data on important agronomic traits were collected. The combined analysis of variance (ANOVA) across years and locations showed highly significant differences among genotypes for all traits, indicating the presence of sufficient variability among the genotypes. Environmental coefficients of variation (ECV), genotypic coefficients of variation (GCV) and Phenotypic coefficient of variation (PCV) ranges of the study are days to 50% maturity (DM, 0.49) to number of seeds per panicle (NSPP, 6.11); DM (0.31) to PAS (16.99) and DM (1.12) to GY (20.86) in the same order. High h^2_{BS} values were observed in hundred seed weight (HSW, 76%), DS (70%), plant height (PH, 65%) and PAS (63). High value of genetic advance as a percentage of mean (GA% µ) was recorded by PAS (27.42%), PH (26.29%) and DS (20.65%) and moderate amount of GA%µ was recorded by HSW (11.78%) and HW (11.08%). High h^2_{BS} coupled with high to moderate GA%µ was reported for PH (65 and 26.29%); HSW (76 and 11.78%); PAS (63 and 27.42%) and DS (70 and 20.65%) indicating PH and HSW are controlled by additive gene action. GY had strong positive genotypic association with HW (0.99) followed by NSPP (0.96). These results suggested that any positive increase in such traits will increase the grain yield. The genotypic path analysis also showed that head weight per plot (HW, 1.96) and PH (0.55), had high and very high positive direct effect, respectively on GY indicating that these traits are the most important yield component traits. Hence, due consideration should be given to these traits while selecting promising lines.

Key words: Correlation coefficient, genetic advance, heritability, path coefficient, sorghum.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the third (area coverage) and fourth (production) most important food crop of the Ethiopia (CSA, 2014). Intermediate agro-ecology sorghum growing areas of the country are characterized by intermediate altitude (1600 to 1900 masl), high annual rain fall (~1000 mm), temperature and

humidity which support the development of several biotic stresses such as leaf and grain diseases. The efficiency of selection in crop improvement depends on the extent and nature of phenotypic and genotypic variability present in different agronomic traits of populations (Arora, 1991). Research work so far done on sorghum in intermediate altitude sorghum growing agro-ecology did not bring significant increase in the yield crop efficiency. Yield, being quantitative in nature is a complex trait with low heritability and depends upon several other components with high heritability (Grafius, 1959). Hence, selection of plants based directly on yield would not be very reliable. Association of characters was also used to determine the strength relationship among variables. Path analysis was made to assess the direct and indirect effects of each trait on grain yield (Dewey and Lu, 1959). The understanding of association between yield and yield related traits allows the breeders to plan the breeding program accordingly. The present study was conducted to study genetic variability and the interrelationships of traits with grain yield.

MATERIALS AND METHODS

A total of 14 sorghum genotypes which were previously developed by pedigree breeding method were used for this experiment. The experiment was conducted at three locations which represented the intermediate agro ecology, namely: Bako, Jimma and Mechara Agricultural Research Centers in 2014 and 2015 main rainy seasons. The experiment was carried out by using a randomized complete block design (RCBD) with three replications. A plot size of 3 rows with 5 m row length and 0.75 m row width was also used to conduct the experiment at national variety trial stage. Sowing was conducted manually, and the seeds were drilled and spaced 0.75 m apart and latter thinned to a spacing of 20 cm between plants. The trial received Di-ammonium Phosphate (DAP) and urea fertilizers at planting and approximately at 35 to 40 days after emergency, respectively on basis of 100 kg ha^{-1}. Data on days to 50% flowering, plant height (cm), days to maturity, grain yield (tones/ha), hundred seed weight (g), head weight per plot (kg), disease score (1-5 scale, where 1=resistance and 5=susceptible), number of seeds per panicle by following the procedures of (Adugna and Bekele, 2013) and overall agronomic aspect (1-5 scale, where 1=excellent and 5=poor), were recorded.

Data analysis

The General Linear Model procedure (PROC GLM) of SAS (SAS, 2008) was used to determine the variations of genotypes. In this analysis, genotypes, locations and years were fitted as a random effect. The data recorded on the aforementioned parameters across locations and years were analyzed using the following linear additive model as outlined by Snedecor and Cochran (1980) and Annicchiarico (2002). Format of combined analysis of variance across location and year is shown in Table 1. The linear statistical model for the combined analysis of experiments laid out in a randomized complete block design is:

$$Xijkl = \mu+G_i+R_{jkl}+L_k+Y_l+GL_{ik}+GY_{il}+LY_{kl}+GLY_{ikl}+E_{ijkl,}$$

where Xijkl = observed value, μ = overall mean, G_i = effect of genotype, R_{jkl} = effect of replication, L_k = effect of location, Y_l = effect of year, $GL_{ik}+GY_{il}+LY_{kl}+GLY_{ikl}$ = effects of Genotype×Location, Genotype×Year, Location×Year, and Genotype×Location×Year interactions, respectively. Eijkl = residual effects or experimental error. Additionally, g, r, l, and y are numbers of genotypes, replications, locations and years, in the same order and g = 14, r = 3, l = 3 and y = 2.

Components of variance, estimation of heritability and genetic advance

The phenotypic and genotypic variances for the combined data across year and location were computed according to the method suggested by Annicchiarico (2002).

$$\sigma^2g = M5-M3-M4+M2/rly$$

where σ^2_g = variance of genotypes.

$$\sigma^2gl = M3-M2/ry$$

where σ^2_{gl} = variance of genotypes by locations interactions.

$$\sigma^2gy = M4-M2/rl$$

where σ^2_{gy} = variance of genotypes by years interactions.

$$\sigma^2gly = M2-M1/r$$

where σ_{gy}^2 = variance of genotypes by location and years interactions.

Phenotypic, genotypic and environmental coefficients of variation

Phenotypic coefficient of variation (PCV), genotypic coefficients of variation (GCV) and environmental coefficients of variation (ECV) were calculated according to Burton (1952) using combined data across the three locations and two years.

$$GCV = \sqrt{(\sigma^2g/\mu)} \times 100$$

$$PCV = \sqrt{(\sigma^2p/\mu)} \times 100$$

$$ECV = \sqrt{(\sigma^2e/\mu)} \times 100$$

Broad sense heritability (h^2_{BS}) for the combined data across year and location was estimated according to Gordon et al. (1972), and it was grouped as low (below 30%), medium (30-60%) and high (above 60%) as suggested by Johnson et al. (1955).

$$h^2BS = \sigma^2g / (\sigma^2g + \sigma^2gy/y + \sigma^2gl/l + \sigma^2gly/ly + \sigma^2/ryl)$$

where r, y and l denote the number of replicates, years and

*Corresponding author. E-mail: adanegy10@gmail.com.

Table 1. Format of combined ANOVA across years and locations used in the present study.

Source of variations	Degree of freedom	Mean squares	Expected mean squares
Locations (L)	l-1	M9	$\sigma^2 + \gamma\sigma^2\rho + \rho\sigma^2\gamma\lambda\psi + \rho\psi\sigma^2\gamma\lambda + \rho\gamma\sigma^2\lambda\psi + \rho\gamma\psi\sigma^2\lambda$
Year (Y)	y-1	M8	$\sigma^2 + \gamma\sigma^2\rho + \rho\sigma^2\gamma\lambda\psi + \rho\lambda\sigma^2\gamma\psi + \rho\gamma\sigma^2\lambda\psi + \rho\gamma\lambda\sigma^2\psi$
Y×L	(y-1)(l-1)	M7	$\sigma^2 + \gamma\sigma^2\rho + \rho\sigma^2\gamma\lambda\psi + \rho\gamma\sigma^2\lambda\psi$
Replications (r)	r-1	M6	$\sigma^2 + \gamma\sigma^2\rho$
Genotypes (G)	g-1	M5	$\sigma^2 + \rho\sigma^2\gamma\lambda\psi + \rho\lambda\sigma^2\gamma\psi + \rho\psi\sigma^2\gamma\psi + \rho\lambda\psi\sigma^2\gamma$
G×Y	(g-1)(y-1)	M4	$\sigma^2 + \rho\sigma^2\gamma\lambda\psi + \rho\lambda\sigma^2\gamma\psi$
G×L	(g-1)(l-1)	M3	$\sigma^2 + \rho\sigma^2\gamma\lambda\psi + \rho\psi\sigma^2\gamma\lambda$
G×L×Y	(g-1)(l-1) (y-1)	M2	$\sigma^2 + \rho\sigma^2\gamma\lambda\psi$
Error	(g-1)(r-1)ly	M1	σ^2

locations, respectively.

Genetic advance (GA) was computed by following the procedure suggested by Johanson et al. (1955).

$$GA = K \times h^2BS \times \sqrt{\sigma^2 p}$$

where K= the selection intensity at 5% (2.06).

Genetic advance as percent of mean [GA (% mean)] computed as follows and it was further sorted out as low (0-10%), moderate (10-20%) and high (≥20%) as given by Johnson et al. (1955) and Falconer and Mackay (1996).

$$GA\ (\%\ of\ mean) = GA/\mu \times 100$$

Correlation and path coefficient analyses

Correlation coefficient was computed from variance and covariance components as suggested by Burton (1952), Wright (1968) and Singh and Chaundhary (1985). The correlation coefficient was further partitioned into direct and indirect causes according to Dewey and Lu (1959), Wright (1960) and Singh and Chaundhary (1985).

RESULTS AND DISCUSSION

The combined analysis of variance across years and locations showed highly significant differences among the genotypes (G) for all traits, which indicates the presence of variability among the genotypes being evaluated and the possibility of ample scope of improvement by selection (Table 2). The G×L interactions of genotypes were significant for all traits except for days to maturity. The significant difference of G×L interactions indicates that genotypes respond differently across location for these traits and this requires testing of genotypes over a range of locations. A highly significant G×L×Y interaction was also observed for most of the traits, showing that genotypes were inconsistent in their performance when tested across locations and years. Similar finding was reported by Phuke et al. (2017). They reported highly significant variation of G and G×Y×L interaction for days to 50% flowering, plant height, hundred seed weight and grain yield on sorghum. Highly significant yield differences between genotypes, locations, year and their interactions show the need to develop genotypes that are adapted to specific environmental conditions and the need to identify genotypes that are exceptionally stable across environments. A large yield and agronomic traits variation explained by genotypes indicated that the genotypes were diverse, with large differences between locations means causing most of the variation of traits.

Insignificant G×L×Y interaction for days to 50% flowering, days to maturity and hundred seed weight indicating genotypes performed similarly across year and locations with respect to these traits. For all traits in the present study, the mean square values of G×L×Y interactions were lower than genotypic value (Table 2), signifying that the traits are mainly under genetic control. Similar finding was reported by Nida et al. (2016) on grain yield of sorghum. G×L interactions were non-significant for days to maturity, significant ($p<0.05$) for hundred seed weight and highly significant ($p<0.01$) for the rest of traits. The significant difference of G×L interactions indicates that genotypes respond differently across location for these traits and this requires testing of genotypes over a range of locations to identify stable genotypes. These results are supported by Khan et al. (2013) who found significant variation of G, G×L and L×Y interactions for plant height in sunflower. Highly significant G×L interactions for days to 50% flowering, plant height and grain yield was also reported by Tadesse et al. (2008) on sorghum parental lines.

Estimates of variance components, heritability and genetic advance

The present results on variance component showed that the phenotypic variances were slightly higher than the genotypic variance for days to 50% flowering, plant height, hundred seed weight, disease score and overall agronomic aspect, signifying the influence of environment on these traits was very low. ECV, GCV and PCV ranges of this study are days to maturity (0.49) to number of seeds per panicle (6.11), days to maturity (0.31) to overall agronomic aspect (16.99) and days to maturity

Table 2. Mean square for agronomic traits of sorghum genotypes tested at Mechara, Bako & Jimma in 2014 & 2015 main rainy seasons.

Source of variation	Degree of freedom	Days to 50% flowering	Plant height (cm)	Days to 50% maturity	Gain yield (tons ha^{-1})	Hundred seed weight (g)	Head weight per plot (kg)	Disease score (1-5)	Number of seeds per panicle	Overall agronomic aspect (1-5)
Locations (L)	2	3619.8**	58259.1**	9468.6**	4.5**	4.5**	105.9**	6.9**	4561187001.0**	6.9**
Year (Y)	1	24702.5**	212628.6**	20773.6**	421.7**	1.9**	1499.9**	6.8**	7996833.0 ^{ns}	14.0**
Y×L	2	1781.0**	49162.5**	21.8 ^{ns}	67.3**	0.5*	101.9**	8.2**	1974642669.0**	3.1**
Replications	2	80.3 ^{ns}	557.9 ^{ns}	18.5^{ns}	0.1^{ns}	0.3^{ns}	3.0 ^{ns}	2.9**	628374733.0**	0.3^{ns}
Genotypes (G)	13	183.0**	22047.5**	64.2**	9.3**	0.6**	13.1**	1.7**	634475393.0**	6.0**
G×Y	26	313.4**	6240.6**	60.7**	9.6**	0.1^{ns}	10.7**	0.2^{ns}	557218242.0**	1.9**
G×L	26	86.8**	1550.6**	26.3^{ns}	2.0**	0.2*	2.9**	0.6**	325777153.0**	1.2**
G×L×Y	13	37.7^{ns}	2642.8**	17.7^{ns}	2.6**	0.1^{ns}	8.4**	0.3*	217382942.0**	0.9**
Error	166	32.0	458.1	13.0	0.2	0.2	1.3	0.2	85887800.0	0.4
CV (%)	-	5.0	11.1	2	12.2	16.7	18.3	21.8	25.9	21.7
Mean	-	114.18	193.49	172.38	4.01	2.29	6.35	2.08	35759.40	2.76
LSD	-	3.7	14.1	2.4	0.3	0.3	0.8	0.3	6099.0	0.42

*, **, ^{ns}Significant at 0.05, 0.01 and non significant, respectively; LSD: The least significant difference value; CV(%): coefficient of variation in percentage.

Table 3. Genetic parameters for agronomic traits of combined data of sorghum lines tested at Mechara, Bako and Jimma in 2014 and 2015 main rainy season.

Genetic parameter	Days to 50% flowering	Plant height (cm)	Days to 50% maturity	Gain yield (tons ha^{-1})	Hundred seed weight (g)	Head weight plot^{-1} (kg)	Disease score (1-5)	Number of seeds per panicle	Overall agronomic aspect (1-5)
PV	28.75	1443.56	3.72	0.70	0.03	1.66	0.09	41507133.3	0.34
GV	9.97	938.84	0.29	0.02	0.02	0.44	0.06	1729836.7	0.22
EV	1.78	25.45	0.72	0.01	0.01	0.08	0.01	4771544.0	0.02
PCV%	4.70	19.64	1.12	20.86	7.56	20.29	14.42	18.02	21.13
GCV%	2.77	15.84	0.31	3.53	6.18	10.45	11.78	3.68	16.99
ECV%	1.17	2.61	0.49	2.49	4.37	4.45	4.81	6.11	5.12
h^2BS (%)	35	65	8	2	76	27	70	4	63
GA	3.83	50.87	0.31	0.04	0.27	0.70	0.43	557.41	0.76
GA%μ	3.36	26.29	0.18	0.95	11.78	11.08	20.65	1.56	27.42
Mean(μ)	114.18	193.49	172.38	4.01	2.29	6.35	2.08	35759.40	2.76

GV: Genotypic variance, EV: environmental variance, PV: phenotypic variance.

(1.12) to grain yield (20.86), in the same order (Table 3). Based on the classification of Sivasubramanian and Madhavamenon (1973), high PCV values were observed in grain yield, head weight and overall agronomic aspect and moderate PCV value were observed in plant height, disease score and number of seeds per plant. Moderate amount of GCV were observed in plant height, head weight, disease score and

overall agronomic aspect. PCV value of genotypes was much higher than GCV for grain yield showing the environments were diverse as a result of this, the response of genotypes were significantly different in each environment. The difference between PCV and GCV was maximum for grain yield followed by number of seeds per panicle, indicating that these traits are more influenced by the environment. The highest PCV and GCV value for plant height is in accordance with the report of Abraha et al. (2015). Tomar et al. (2012) and Godbharle et al. (2010) also reported low PCV and GCV on days to 50% flowering and Warkad et al. (2008) and Abraha et al. (2015) also reported low PCV and GCV on days to maturity. Similarly, moderate value of PCV and GCV on plant height reported by Warkad et al. (2008) agrees with present research report.

The GCV is only an indication of the presence of high degree of genetic variation; however, the amount of heritable portion of variation can only be determined with the help of estimates of heritability and genetic advance. Broad heritability (h^2BS) for the combined data across year and location was estimated according to Gordon et al. (1972) and it was further grouped as low (below 30%), medium (30-60%) and high (above 60%) as suggested by Johnson et al. (1955). Based on the aforementioned classification, high heritability values were observed in hundred seed weight (76%), disease score (70%), plant height (65%) and over all agronomic aspect (63) and moderate value of heritability was observed in days to 50% flowering (35%). High heritability of the traits indicates that they are less influenced by environment in their phenotypic expression. Therefore, the breeders could get chance to select promising genotypes based on the phenotypic performance of these traits. Agreeing with the present study, high heritability estimates for overall agronomic aspect were reported by Abraha et al. (2015). High heritability estimates for plant height was also reported by Tomar et al. (2012), Godbharle et al. (2010) and Bhagasara et al. (2017) which is in accordance with the present findings. Like the present study, high heritability estimates for hundred seed weight was also reported by Bhagasara et al. (2017).

On the other hand, low heritability was observed for days to maturity (8%), grain yield (2%), head weight (27%) and number of seed per panicle (4%) indicating that these traits would not respond to phenotypic selection. Low heritability for grain yield were also reported by Bello et al. (2001), Bello et al. (2007), Naim et al. (2012) and Abraha et al. (2015) which is in agreement with the present study. Furthermore, in the present finding, Naim et al. (2012) reported low heritability for head weight and number of seed per panicle.

The heritability values alone provide no indication of the amount of genetic progress that would result in selecting the best individual, but heritability estimates along with the genetic advance are more useful (Johnson et al., 1955). Genetic advance as percent mean [GA (% mean)] sorted out as low (0-10%), moderate (10-20%) and high (≥20%) as given by Johnson et al. (1955) and Falconer and Mackay (1996). Thus, in the present study high value of GA%μ was recorded by overall agronomic aspect (27.42%), plant height (26.29%) and disease score (20.65%) and moderate amount of GA%μ was recorded by hundred seed weight (11.78%) and head weight (11.08%). On the other hand, low amount of GA%μ was recorded by days to maturity (0.18%), grain yield (0.95%) and number of seed per panicle (1.56%) and days to 50% flowering (3.36%) in the same order. High heritability coupled with high to moderate genetic advance as percent of mean was reported for plant height (65 and 26.29%), hundred seed weight (76 and 11.78%), overall agronomic aspect (63 and 27.42%) and disease score (70 and 20.65%) in the same order. These indicate that plant height and hundred seed weight are controlled by additive gene action. Therefore, the phenotypic selection based on these traits would result in the improvement of the genotypes. Similar finding of high heritability coupled with high to moderate genetic advance as percent of mean was reported by Sharma et al. (2006) and Ranjith et al. (2017) for hundred seed weight; Arunkumar et al. (2004), Godbharle et al. (2010), Tomar et al. (2012), Kour and Pradhan, (2016), and Ranjith et al. (2017) for plant height. On the other hand, moderate value of heritability along with low genetic advance as percent of mean was observed for days to 50% flowering indicating that variability is mainly due to the non-additive gene effects and hence heterosis breeding can be successfully exploited in improving this character.

Phenotypic, genotypic and environmental correlation coefficients

The results of genotypic correlation coefficient were higher than those of phenotypic and environmental correlation coefficients for all the characters except the genotypic association of days to 50% flowering with grain yield and disease score which revealed that there was a greater contribution of genetic factors in the expression of these traits in relation to the environmental factor (Table 4). Grain yield had strong positive genotypic association with head weight (0.99) followed by number of seeds per panicle (0.96). These results suggested that any positive increase in such traits will increase the grain yield. Similar findings of strong positive grain yield association with number of seeds per panicle and head weight were reported by Tourchi and Rezai (1997), by Tesso et al. (2011) with head weight and by Yang and Yang (1995) with number of seeds per panicle. The genotypic association also showed that hundred seed weight had strong positive association with disease score (0.72), indicating that small seeded genotypes are more resistance to disease reaction that is why during data

Table 4. Phenotypic (r_p), genotypic (r_g) and environmental (r_e) correlation coefficients of various traits for the combined data.

Correlation		PH	DM	GY	HSW	HW	DS	NSPP	PAS
	r_p	0.49*	0.60**	-0.36*	-0.20^{NS}	-0.42*	-0.07^{NS}	-0.06^{NS}	0.20^{NS}
DF	r_g	0.73**	0.65**	-0.36*	-0.28^{NS}	-0.78**	-0.05^{NS}	0.12^{NS}	0.43*
	r_e	0.16^{NS}	0.22^{NS}	-0.23^{NS}	-0.10^{NS}	-0.10^{NS}	-0.03^{NS}	-0.09^{NS}	-0.08^{NS}
	r_p	1	0.55**	-0.43*	0.01^{NS}	-0.57**	0.21^{NS}	-0.26^{NS}	0.59**
PH	r_g	1	0.75**	-0.64**	0.03^{NS}	-0.91**	0.28^{NS}	-0.44*	0.72**
	r_e	1	0.17^{NS}	-0.04^{NS}	0.10^{NS}	0.03^{NS}	0.06^{NS}	-0.11^{NS}	0.07^{NS}
	r_p		1	-0.41*	0.28^{NS}	-0.35*	0.31^{NS}	-0.52**	0.61**
DM	r_g		1	-0.49*	0.63**	-0.46*	0.54**	-0.92**	0.89**
	r_e		1	-0.32*	0.01^{NS}	-0.16^{NS}	-0.02^{NS}	-0.20^{NS}	0.10^{NS}
	r_p			1	0.02^{NS}	0.93**	-0.61**	0.78**	-0.80**
GY	r_g			1	-0.27^{NS}	0.99**	-0.97**	0.96**	-0.99**
	r_e			1	0.08^{NS}	0.64**	0.05^{NS}	0.52**	-0.25^{NS}
	r_p				1	0.21^{NS}	0.51*	-0.48*	$0.3\ 1^{NS}$
HSW	r_g				1	0.15^{NS}	0.72**	-0.70**	0.50*
	r_e				1	0.03^{NS}	-0.03^{NS}	-0.35*	0.05^{NS}
	r_p					1	-0.45*	0.58**	-0.74**
HW	r_g					1	-0.81**	0.77**	-0.99**
	r_e					1	0.09^{NS}	0.26^{NS}	-0.24^{NS}
	r_p						1	-0.77**	0.81**
DS	r_g						1	-0.99**	0.99**
	r_e						1	0.08^{NS}	0.10^{NS}
	r_p							1	-0.80**
NSPP	r_g							1	-0.99**
	r_e							1	-0.13^{NS}

^{NS}, * , ** and are no significant, significant at 0.05 and 0.01 probability level, respectively.

collection, 1 is assigned for resistance and 5 is for susceptible genotypes. In the present study, plant height had positive significant genotypic association with days to flowering (0.73) showing that late blooming genotypes are taller than early blooming ones. This result agrees with research findings of Murray et al. (2008), Bunphan et al. (2014), and Abraha et al. (2015). In the present study, days to 50% flowering has also positive significant genotypic association with overall agronomic aspect (0.43) showing that early blooming genotypes were preferred by sorghum breeder during evaluation.

On the other hand, the genotypic association showed that the overall agronomic aspect and disease score had strong negative association with grain yield, indicating that disease resistance genotypes and genotypes with excellent in overall agronomic aspect gave better yield that is why during data collection, 1 is assigned for excellent genotypes and 5 is for poor genotypes. Similarly, overall agronomic aspect had the strongest negative genotypic association with grain yield (-0.99), head weight (-0.99) and number of seeds per panicle (-0.99), showing that high yielding along with big panicle and high number of seeds per panicle are a good parameter to select a genotype of excellent in agronomic desirability (Table 4).

Path coefficient analysis

In crop improvement, information on the association between two traits is necessary to improve the simultaneous selection of traits. However, evaluating and interpreting the amount an association can lead to mistakes in the selection strategy due to pleiotropism. As a result of this reason, investigating the cause and effect of the relationships into direct and indirect effects of a

Table 5. Genotypic path coefficient analysis direct effects on main diagonal (bold & diagonal) and indirect effects (off diagonal) of different agronomic traits on grain yield of sorghum genotypes.

Correlation	DF	PH	DM	HSW	HW	NSPP	r_g
DF	**-0.70**	0.38	0.99	0.50	-1.53	-0.01	-0.36
PH	-0.51	**0.53**	1.15	-0.05	-1.78	0.04	-0.64
DM	-0.46	0.39	**1.53**	-1.14	-0.90	0.08	-0.49
HSW	0.19	0.02	0.96	**-1.80**	0.29	0.06	-0.27
HW	0.55	-0.48	-0.70	-0.27	**1.96**	-0.07	0.99
NSPP	-0.08	-0.23	-1.41	1.26	1.51	**-0.09**	0.96

DF: Days to 50% flowering, PH: Plant height in cm, DM: days to 50% maturity, GY: grain yield in ton/ha, HSW: hundred seed weight (g), HW: head weight per plot (kg), NSPP: Number of seeds per panicle, r_g: genotypic correlation coefficients with grain yield.

group of traits over the dependent variable by path analysis is very important (Cruz et al., 2004).

Path coefficients were classified as suggested by Lenka and Mishra (1973), where, 0.00-0.09 is negligible association effects, 0.10-0.19 is low, 0.20-0.29 is moderate, 0.30-0.99 is high and >1.0 is very high. Accordingly, the genotypic direct effect of plant height on grain yield was high and positive (0.55) but their genotypic correlation coefficient was negative (-0.64) and it was mostly due to very high positive indirect effects via days to maturity (1.13). Similarly, genotypic direct effect of days to maturity on grain yield was very high and positive (1.53) but their genotypic correlation coefficient was negative (-0.49) this is due to very high negative indirect effects via hundred seed weight (-1.14), and high negative indirect effects via head weight (-0.90) and days to 50% flowering (-0.46). The genotypic path analysis (Table 5) also showed that head weight had very high positive direct effect on grain yield (1.96) indicating the importance of head weight as one of the most important yield component traits. Hence, due consideration should be given to traits like head weight and plant height, while planning a breeding strategy for increased grain yield and promising lines could be selected based on these traits. High and positive direct effect of head weight on grain yield was reported by Ezeaku and Mohammed (2006).

Conclusions

The present study showed that plant height (PH) and hundred seed weight (HSW) are controlled by additive gene action; thus, the phenotypic selection based on these traits would result in the improvement of the genotypes. On the other hand, low heritability traits like grain yield (GY) are greatly influenced by the environment and are suggested either to be tested over a wide range of environments or could be selected using molecular markers linked to QTLs for the target traits that enables individuals to be scored based on their genetic makeup and their phenotypic performance. The present studies also showed that the GY of sorghum genotypes can be increased by selecting head weight (HW) and number of seeds per panicle jointly. It could also be concluded that selection of short plants will favor a higher yield (negative correlation). The genotypic path analysis also showed that plant height and head weight had high positive direct effect on grain yield indicating these traits are the most important yield component traits. Hence, due consideration should be given to these traits while selecting promising lines.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abraha T, Githiri SM, Kasili R, Araia W, Nyende AB (2015). Genetic variation among sorghum (*Sorghum bicolor* L. Moench) landraces from Eritrea under post-flowering drought stress conditions. American Journal of Plant Sciences 6:1410-1424.

Adugna A, Bekele E (2013). Morphology and fitness components of wild × crop F1 hybrids of *Sorghum bicolor* (L.) in Ethiopia: Implications for survival and introgression of crop genes in the wild pool. Plant Genetic Resources 11(3):196-205.

Annicchiarico P (2002). Genotype × Environment Interactions: Challenges and Opportunities for Plant Breeding and Cultivar Recommendations. Food and Agriculture Organization of the United Nations. ISBN: 9251048703. P. 126.

Arora PP (1991). Genetic Variability and its Relevance in Chickpea Improvement. International Chickpea News Letter 25:9-10.

Arunkumar B, Biradar BD, Salimath PM (2004). Genetic variability and character association studies in rabi sorghum. Karnataka Journal of Agricultural Sciences 17(3):471-475.

Bello D, Kadams AM, Simon SY (2001). Correlation and path coefficient analysis of grain yield and its components in sorghum. Nigerian Journal of Tropical Agriculture 3:4-9.

Bello D, Kadams AM, Simon SY and Mashi DS (2007). Studies on genetic variability in cultivated sorghum (*Sorghum bicolor L. Moench*) cultivars of Adamawa State Nigeria. American-Eurasian Journal Of Agricultural and Environmental Sciences 2:297-302.

Bhagasara VK, Ranwah BR, Meena BL and Khan R (2017). Estimation of GCV, PCV, heritability and genetic gain for yield and its related components in sorghum [*Sorghum bicolor* (l.) Moench]. International Journal of Current Microbiology and Applied Sciences 6(5):1015-1024.

Bunphan D, Jaisil P, Sanitchon J (2014). Genetic variation and correlation of some agronomic traits, biomass and ethanol yield in diverse sweet sorghum *(Sorghum bicolor* L*. Moench)* cultivars*.* Khon Kaen Agricultural Journal 42(4):605-616.

Burton GW (1952). Quantitative Inheritance in Grasses. Proceedings of the 6th International Grass land Congress 1:227-283.
Cruz CD, Regazzi AJ, Carneiro AJ, Souza PC (2004). Modelos Biométricos Aplicados Ao Melhoramento Genético. Vol 2. 3rd Edition UFV, Viçosa.
Central Statistical Agency (CSA) (2014). Agricultural Sample Survey Report on Area and Production for Major Crops (Private Peasant Holdings Meher Season Statistical Bulletin number 532, Addis Ababa, Ethiopia. . 14.
Dewy DR, Lu KH (1959). A correlation and path coefficient analysis of crested wheat grass seed production. Agronomy Journal 51:515-518.
Falconer DS, Mackay FC (1996). Introduction to Quantitative Genetics. Longman, New York. P. 464.
Godbharle AR, More AW, Ambekar SS (2010). Genetic variability and correlation studies in elite 'B' and 'R' lines in kharif sorghum. Electronic Journal of Plant Breeding 1(4):989-993.
Gordon IL, Byth PE, Balam LN (1972). Variance of heritability ratios estimated from phenotypic variance components. Biometric 28:401-415.
Grafius JE (1959). Heterosis in barley. Agronomy Journal 51: 551-554.
Johnson HW, Robinson HF, Comstock RE (1955). Estimates of genetic and environmental variability in soybeans. Agronomy Journal. 47: 314-318.
Khan H, Rehman HU, Bakht J, Khan SA, Hussain I, Khan A, Ali S (2013). Genotype × environment interaction and heritability estimates for some agronomic characters in sunflower. The Journal of Animal and Plant Sciences 23(4):1177-1184.
Kour S, Pradhan UK (2016). Genetic variability, heritability & expected genetic advance for yield & yield components in forage sorghum [*Sorghum bicolor* (L.) Moench]. Rashi 1(2):71-76.
Lenka D, Mishra B (1973). Path coefficient analysis of yield in rice varieties. Indian Journal of Agricultural Science 43:376-379.
Murray SC, Rooney WL, Mitchell SE, Sharma A, Klein PE, Mullet JE, Kresovich S (2008). Genetic improvement of sorghum as a biofuel feedstock: II. QTL for stem and leaf structural carbohydrates. Crop Science 48:2180-2193.
Naim EAM, Ibrahim IM, Mohammed EAR, Elshiekh AI (2012). Evaluation of some local sorghum (*Sorghum bicolor L.* Moench) genotypes in rain-fed. International Journal of Plant Research 2:15-20.
Nida H, Seyoum A, Gebreyohannes A (2016). Evaluation of yield performance of intermediate altitude sorghum (*Sorghum bicolor* (L.) *Moench*) genotypes using genotype × environment interaction analysis and GGE biplot in Ethiopia. International Journal of Trend in Research and Development 3(2):2394-9333.
Phuke RM, Anuradha K, Radhika K, Jabeen F, Anuradha G, Ramesh T, Hariprasanna K, Shivaji P, Mehtre P, Deshpande SP, Anil G, Das RR, Rathore A, Hash T, Reddy BVS, Kumar AA (2017). Genetic variability, genotype × environment interaction, correlation, and GGE bplot analysis for grain iron and zinc concentration and other agronomic traits in RIL population of sorghum (*Sorghum bicolor* L. *Moench*). Frontiers in Plant Science 8:1-13.
Ranjith P, Ghorade RB, Kalpande VV, Dange AM (2017). Genetic variability, heritability and genetic advance for grain yield and yield components in sorghum. International Journal of Farm Sciences 7(1):90-93.
SAS Institute Inc (2008). The SAS System for Windows. Version 9.1. Cary, NC: SAS Institute Inc.USA.
Sharma H, Jain DK, Sharma V (2006). Genetic Variability and Path Coefficient Analysis in Sorghum. Indian Journal Of Agricultural Research 40(4):310-312.
Singh RK, Chaudhary BD (1985). Biometrical Methods in Quantitative Genetics Analysis. Kalyani Pupl., New Delhi.
Sivasubramanian S, Madhavamenon P (1973). Genotypic and phenotypic variability in rice. The Madras Agricultural Journal 60:1093-1096.
Snedecor DC, Cochran WG (1980). Statistical Methods 7ed. The Iowa State Univ. Press, Ames.
Tadesse T, Tesso T, Ejeta G (2008). Combining ability of introduced sorghum parental lines for major morpho-agronomic traits. Journal of SAT Agricultural Research 6:1-7.
Taurchi M, Rezai AM (1997). Correlation between traits and path analyzed for grain yield in sorghum. Iranian Journal of Agricultural Sciences. 28(1):73-86.
Tesso T, Tirfessa A, Mohammed H (2011). Association between morphological traits and yield components in the durra sorghums of Ethiopia. Hereditas 148:98-109.
Tomar SS, Sivakumar S, Ganesamurthy K (2012). Genetic variability and heritability studies for different quantitative traits in sweet sorghum [*Sorghum bicolor* (L.) Moench] genotypes. Electronic Journal of Plant Breeding 3(2):806-810.
Warkad YN, Potdukhe NR, Dethe AM, Kahate PA, Kotgire RR (2008). Genetic variability, heritability and genetic advance for quantitative traits in sorghum germplasm. Agricultural Science Digest 28(3):165-169.
Wright S (1960). Path coefficient and path regression: alternative or complementary concepts? Biometrics 16:189-202.
Wright S (1968). Evolution and the genetics of populations 1. Genetics and Biometrics Foundations. The University of Chicago.
Yang W, Yang WG (1995). Studies on the genetic correlation of important traits of sweet sorghum. Journal of Tilin-Agricultural University 17:29-31.

.

Vol. 13(49), pp. 2788-2797, 6 December, 2018
DOI: 10.5897/AJAR2017.12957
Article Number: 6AA060C59494
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic variability of some yield and yield related traits in recombinant inbred lines of tef [*Eragrostis tef* (Zucc.) Trotter] at Laelay Maichew District, Northern Ethiopia

Haftamu Hailekiros

Plant Breeding Department, Axum Agricultural Research Center, P. O. Box 230, Axum, Ethiopia.

Received 27 December, 2017; Accepted 15 February, 2018

Tef [*Eragrostis tef* (Zucc.) Trotter] is a tetraploid almanac plant which belongs to the grass family, Poaceae and plays a vital role in the Ethiopian national food. In this study, thirty-four F_2 derived F_7 recombinant tef inbred lines, two standard (Kora and Quncho) and one local checks were field evaluated for genetic variability in grain yield and yield related characters at Axum Agricultural Research Center in 2014 cropping season. Triplicated randomized complete block design was used. Data were collected on fourteen yield and yield related traits and the analysis of variance reveled that genotypes varied significantly for all traits studied except thousand kernel weight. Highest genotypic coefficient of variation (GCV) was computed for biomass yield followed by panicle yield, plant height and grain yield, in contrast, lowest GCV was noted for number of fertile tillers per plant, days to heading, days to maturity and lodging index, whereas the highest phenotypic coefficient of variation (PCV) was recorded for panicle yield, plant height, biomass production rate per day and biomass yield. The highest broad sense heritability values were recorded for plant height, biomass production rate per day, biomass yield, days to 50% heading and grain yield. The highest genetic advance as percent of mean was recorded for biomass production rate per day, biomass yield, grain yield and grain yield production rate per day, while the lowest genetic advance as a percent of mean was computed for number of productive tillers per plant, panicle length, days to heading and panicle weight. The overall study indicated that there were variations in magnitude of variability in traits for the genotypes studied which showed smooth selection for further improvement in tef.

Key words: Genotypic coefficient of variation (GCV), genetic advance, heritability, phenotypic coefficient of variation (PCV), seed yield, variability.

INTRODUCTION

Tef [(*Eragrostis tef* (Zucc.(Troter) 2n = 4x = 40)] is a tetraploid plant, belonging to the family Poaceae, genus Eragrostis which comprises about 350 species (Watson and Dallawitz, 1992). The center of origin and diversity of

E-mail: haftamumeshesha12@gmail.com.

the tef crop is Ethiopia (Vavilov, 1951).

Tef was possibly cultivated in Ethiopia even before the introduction of emmer wheat and barley (Ebba, 1975). Early investigations of diversity showed a huge variability in majority of the traits studied in more than 100 panicle sample collections from different agro ecologies of Ethiopia (Mengesha et al., 1965). Later, Ebba (1975) characterized 35 distinct tef ecotypes and classified them based on phenology and plant morphology.

Tef cultivation as a cereal food grain is restricted to Ethiopia with an annual cultivation on 3.02 million hectares of land and a total production of 4.4 million tons with the national average standing at 1.46 t/ha (CSA, 2014). Tef is ecologically and agronomically versatile crop. It can be grown from below sea level to 3000 m above sea level, under various rainfall, temperature and soil regimes.

Tef is the most preferred crop as source of food and feed in Ethiopia. Besides, it is tolerant to drought, water-logging, and pests particularly against storage pests. Nowadays, tef has become a globally popular crop for its glutein free property that makes it conducive for people suffering from celiac disease and diabetic because of its slow release of carbohydrates.

Hence, it is regarded as a promising alternative food replacing gluten-containing cereals like wheat, barley and rye in products such as pasta, bread, beer, cookies and pancakes (Spaenij-Dekking et al., 2005). Recently, Cannarozzi et al. (2014) supported this fact with results from the genome sequence initiative. Tef has high iron content that makes it appropriate for pregnancy-related anemia (Alaunyte et al., 2012). The iron content mainly seems to play an essential role in Ethiopia, as there is absence of anemia in areas of tef consumption (BoSTID, 1996).

Despite its greater economic value and large area coverage, tef productivity is much lower as compared to its estimated potential yield level of 6 ton/ha (Ketema, 1993). The low national or regional tef productivity is mainly attributed to susceptibility to lodging, low yield of landraces under widespread cultivation, reduced agronomic management practices, biotic and environmental stresses (Ketema, 1997; Assefa et al., 2011). However, no variability has been studied on tef genotypes in the area. Hence, evaluation of different genotypes of tef is crucial for effective selection.

Generating information and understanding the nature and magnitude of variation existing among tef genotypes is a vital component of improvement programs because it provides evidence on the genetic variability of the crop and sets a base for stratified sampling of breeding populations. Tef represents a unique biodiversity component in the agriculture and food security of millions of farmers in Ethiopia. The conservation, characterization and utilization of the existing tef genetic variability are becoming increasingly important in view of the developing desires and various challenges of small-scale farmers in Ethiopia. This is mainly because tef has remarkable genetic traits valuable for most Ethiopian farmers to cope with erratic climatic conditions, income generation for household and fulfilling concerns of food and nutritional security. Moreover, the conservation and utilization of the tef genetic resources offer a reliable basis for enhancing food security and developing crop diversification in the moisture stress and challenging agro-ecological areas of the district.

Here, an overview of the results of information generated on genetic variability for important yield and yield related traits were presented, which would help to better understand the variability at morphological level and utilize these variability in improving the crop for future breeding program through selection. In view of these, the present study was carried out with the aims to assess the characters of both genotypic and phenotypic variability and to estimate broad sense heritability (H) and genetic advance expectations from selection of the different traits.

MATERIALS AND METHODS

Description of the study area

The experiment was carried out at Axum Agricultural Research Center of Tigray Agricultural Research Institute (TARI) with rainfall during 2014 main cropping season. Axum Agricultural Research Center (AxARC) is suited in the northern part of Ethiopia, 1024 km North of Addis Ababa. It lies at latitude 13° 15’ N and longitude 38 ° 34’ E. It has an altitude of 2148 m.a.s.l and it receives a monomodal unevenly distributed average annual rainfall of 756.9 mm per annum. The long term mean minimum and maximum temperature is 11.2 and 27.8° C, respectively. The soil type of the study area is classified as vertisol with a pH of 7.5 to 8.3 (AxARC unpublished, 2012).

Experimental materials

Thirty four recombinant inbred lines (RILS) of tef together with two released variety (Quncho and Kora) and one local check were used in the study. The 34 RILs were randomly taken from hundreds of RILs at the seventh filial generation from the National Tef Research Project of Debre Zeit Agricultural Research Center (DZARC).

Experimental design and field management

The test tef genotypes were laid out in triplicated randomized complete blocks design of plots comprised of six rows of 2.5 m length and 1.2 m width (3 m^2) standard plot size for variety trial with 0.2 m of row spacing. The spaces between plots and replications were 1 and 1.5 m, respectively. Sowing was done by manual drilling along the rows at a seed rate of 1.5 g per row on the basis of 25 kg/ha recommended rate. The source of P_2O_5 and N were DAP and urea, respectively, both applied at the rate of 100 kg ha^{-1}. All the DAP was applied at planting and urea was applied in two splits, half at the time of planting and the remaining half at tillering stage. The experimental materials were sown on the first week of July 2014, main production season. All other pre and post-planting management practices were done in accordance with the research recommendations for tef production in the area.

Data collection

Data were obtained from fourteen quantitative traits based on plant and plot bases. Data on days to heading, days to maturity, biomass yield, grain yield, harvest index and lodging index were recorded on plot basis from the four middle rows. Derived data like harvest index, biomass production rate per day and grain yield production rate per day was calculated as a ratio of grain yield to shoot biomass, above ground biomass yield to days to physiological maturity and grain yield to physiological maturity, respectively. On the other hand, plant height, panicle length, panicle weight, number of fertile titters per plant and thousand kernel weight were measured on previously selected and tagged ten random samples of plants from the central four middle rows of each plot. Mean values of the ten random samples of plants per plot of the four middle rows were then used for the analyses of data collected on individual plant basis.

Data analysis

Analysis of variance was done using the procedures outlined by Gomez and Gomez (1984) with the help of SAS Computer Statistical Package version 9.1.3 (SAS Institute Inc., 2004) and variance effects were considered as significant and highly significant at $P < 0.05$ and $P < 0.01$, respectively.

Genotypic and phenotypic variance and coefficient of variation

The phenotypic and genotypic variance and coefficient of variation was estimated according to the method suggested by Burton and DeVane (1953) as follows:

$\square^2_g = \frac{Mg - Me}{r}$

Where, σ^2_p = phenotypic variance; σ^2_g = genotypic variance; σ^2_e = environmental variance (error mean square); M*g* = mean sum square of genotypes; M*e* = mean sum square of error; r = number of replications.

Phenotypic coefficient of variation, PCV = $\frac{\sqrt{\sigma^2 p}}{\bar{x}} * 100$

Genotypic coefficient of variation, GCV = $\frac{\sqrt{\sigma^2 g}}{\bar{x}} * 100$

Where $\bar{x}$ = population mean.

Estimation of heritability in the broad sense

Heritability in broad sense was computed for each character as suggested by Allard (1960) as:

$H^2 = \frac{\sigma^2 g}{\sigma^2 p} * 100$

Where, $\sigma^2 p$ = phenotypic variance, $\sigma^2 g$ = genotypic variance,

$\sigma^2 p = \sigma^2 g + \sigma^2 e$

Where, σ^2_p = phenotypic variance; σ^2_g = genotypic variance; σ^2_e = environmental variance (error mean square).

Estimation of expected genetic advance

The genetic advance (GA) for selection intensity (K) at 5% was calculated using the formula suggested by Allard (1960) as:

GA = (K) (σp) (h^2)

Where, GA = expected genetic advance, σp = the phenotypic standard deviation, H^2 = heritability in broad sense, K = selection differential (K = 2.06 at 5% selection intensity).

GA (as % of the mean) = $\frac{GA}{\bar{x}} \times 100$

Where, $\bar{x}$ = population mean.

RESULTS AND DISCUSSION

Analysis of variance

Results of the analysis of variance revealed that the mean squares for genotypes were highly significant ($p<0.01$), for all traits studied except thousand kernel weight (Table 1). The range for seed yield per panicle was 1.5 to 14.5 g with mean value of 7.62 g (Table 2) indicating the presence of adequate variations among the tested genotypes. The value of coefficient of variation for most of the traits indicated good precision of the experiment. All the traits scored more than 50% estimate of R^2 except thousand kernel weight (39.24%), showing the adequacy of the model in explaining the variation. In line with the current finding, Tefera et al. (2003a) reported the significant performance difference of 118 recombinant inbred lines (RILs) for days to heading, plant height, days to maturity, panicle height, panicle weight, panicle yield, lodging index, biomass yield and seed yield. Likewise, Debebe et al. (2013) observed significant difference ($P \leq 0.01$) for days to maturity, days to heading, biomass yield, seed yield, harvest index and lodging index.

Mean yield and yield component performance

As indicated in Table 3, the genotypes showed variation in phenology for days to heading ranging from 54 to 64.33 with a mean of 59.87 and days to maturity ranging from 101.67 to 117.67 with a mean of 108.1. The result showed the presence of relatively wide range of variations among the genotypes for maturity. Plaza et al. (2013) also reported wide range of variation among tef genotypes for days to heading and days to maturity with values for days to heading and days to maturity ranging from 58 to 90 days and 83 to 123 days, respectively.

Assefa et al. (2001a) also reported that days to heading and maturity ranged from 25 to 81 and 60 to 140,

Table 1. Description of thirty four RILs, two standard checks and one local used during the study.

Entry	Stock ID	Pedigree
1	RIL#10A	Dz-cr-387 (Quncho) x Dz-01-974 (Dukem)
2	RIL#13A	Dz-cr-387 x Dz-01-974
3	RIL#3A	Dz-cr-387 x Dz-01-974
4	RIL#65A	Dz-cr-387 x Dz-01-974
5	RIL#68A	Dz-cr-387 x Dz-01-974
6	RIL#17A	Dz-cr-387 x Dz-01-974
7	RIL#48A	Dz-cr-387 x Dz-01-974
8	RIL#19A	Dz-cr-387 x Dz-01-974
9	RIL#124A	Dz-cr-387 x Dz-01-974
10	RIL#70A	Dz-cr-387 x Dz-01-974
11	RIL#110A	Dz-cr-387 x Dz-01-974
12	RIL#121A	Dz-cr-387 x Dz-01-974
13	RIL#63A	Dz-cr-387 x Dz-01-974
14	RIL#16A	Dz-cr-387 x Dz-01-974
15	RIL#44A	Dz-cr-387 x Dz-01-974
16	RIL#50B	Dz-cr-387 x Dz-01-974
17	RIL#75B	Dz-cr-387 x Dz-01-974
18	RIL#57B	Dz-cr-387 x Dz-01-97
19	RIL#11B	Dz-cr-387 x Dz-01-974
20	RIL#5B	Dz-cr-387 x Dz-01-974
21	RIL#8B	Dz-cr-387 x Dz-01-974
22	RIL#44B	Dz-cr-387 x Dz-01-974
23	RIL#124B	Dz-cr-387 x Dz-01-974
24	RIL#113B	Dz-cr-387 x Dz-01-974
25	RIL#28B	Dz-cr-387 x Dz-01-974
26	RIL#19B	Dz-cr-387 x Dz-01-974
27	RIL#17B	Dz-cr-387 x Dz-01-974
28	RIL#45B	Dz-cr-387 x Dz-01-974
29	RIL#11C	Dz-cr-387 x Dz-01-974
30	RIL#46C	Dz-cr-387 x Dz-01-974
31	RIL#74C	Dz-cr-387 x Dz-01-974
32	RIL#3C	Dz-cr-387 x Dz-01-974
33	RIL#11D	Dz-cr-387 x Dz-01-974
34	RIL#11E	Dz-cr-387 x Dz-01-974
35	Stand. Check	Quncho (Dz-cr-387)
36	Stand. Check	Kora
37	Local check	Tsaeda zezew

respectively.

Among the genotypes, RIL#44A, with a maturity period of 101.6 days was found to be the earliest, while RIL#44C, with a maturity period of 117.67 days was found to be the latest. Among 37 genotypes, 56.7% showed days to maturity below the grand mean, signifying earliness of these genotypes in their maturity period as compared to the others. On the other hand, as compared to the standard check variety (Quncho) 5.4% of the genotypes showed early maturity. This suggested the higher chance of selecting early genotypes which can tolerate terminal moisture stress, which is one of the bottleneck for tef production in the study area.

In this experiment, genotypes with early heading did not show early maturity and late maturing ones did not necessarily correspond with lateness in days to heading. The result is similar to previous works of Plaza et al. (2013) and Khan (2013) who in that order in tef and wheat reported that the two traits were not similar for most of the studied materials. This might be due to the genetic factors carried by the genotypes for each trait as well as the differences of growing seasons and environments under which the materials were evaluated.

Minimum and maximum plant heights of 98.7 and

Table 2. Analysis of variance results for 14 traits of tef RILs studied.

Traits	Source of variation				
	Replications (df=2)	Genotypes (df=36)	Error (df=72)	CV (%)	R^2 (%)
Days to heading	0.009^{ns}	17.34**	1.194	1.83	87.89
Days to maturity	3.93*	75.78**	1.002	0.93	97.43
No. tillers/plant	0.93*	0.16**	0.084	19.54	55.96
Plant height (cm)	25.33*	71.16**	7.192	2.48	83.45
Panicle length (cm)	44.2**	9.78**	3.776	4.58	61.82
Panicle weight (g)	0.059^{ns}	0.05**	0.021	10.93	53.20
Panicle yield (g)	0.001^{ns}	0.03**	0.010	12.42	63.30
Thousand-kernel weight (g)	0.0033^{ns}	0.01^{ns}	0.005	21.07	39.24
Biomass yield (kg ha^{-1})	123382.8^{ns}	4428863.5**	158686.1	4.75	93.32
Grain yield (kg ha^{-1})	29663.04^{ns}	216104.7**	15425.341	5.23	87.59
Harvest index (%)	7.88*	10.254**	1.583	4.42	77.15
Lodging index (%)	6.387^{ns}	64.417**	6.424	2.96	83.44
Biomass production rate (kg ha^{-1} day^{-1})	15.87^{ns}	410.293**	14.331	4.87	93.48
Grain yield production rate (kg ha^{-1} day^{-1})	1.357^{ns}	22.632**	1.419	5.41	88.88

df = Degrees of freedom, *,** and ns, significant at P ≤0.05, P ≤0.01 and non-significant, respectively, CV (%) = coefficient of variation, R^2 = coefficient of determination and RILs = recombinant inbred lines.

118.33 cm were recorded for RIL#17B and RIL#44B, respectively, the mean value for plant height being 108.22 cm, RIL#3A RIL#46B and RIL#13A, showed longer plant height than the standard check, Quncho. The variation with respect to number of productive tillers per plant for tested genotypes ranged from 1.17 for RIL#17B to 2.07 for Tsaeda-Zezew (Local check). Hence, the local RIL#10A (1147.8 kg ha^{-1}). With regard to biomass yield, 32.4% of the genotypes exceeded the overall mean (8393.5 kg ha^{-1}) of the genotypes while genotypes exceeded 27 and 5.4% of the standard checks, Kora and Quncho, respectively. Thus, there is plenty of variability among the genotypes for selection designed for improvement of this trait.

The range for panicle weight was from 16.7 for RIL#70 to 11.03 for RIL#124A. Thus, 29.7% of the genotypes recorded higher panicle weight than the standard check, Quncho which is the most popular variety currently under production in the area. Therefore, these genotypes can be considered as source materials when increment of this parameter through breeding is needed. The mean value of panicle yield was 7.42 g, RIL#5B, RIL#48A, RIL#70A, RIL#65A and RIL#3A showed superiority for panicle yield than others. Consequently, progress of this trait can be more effective when those genotypes are considered and used in the improvement program. The computed harvest index for genotypes ranged from 22.9% for RIL#11E to 32% for RIL#11C. Genotypes RIL#11C, RIL#13A, RIL#44A and RIL#28B had greater values for harvest index than even the standard checks Kora and Quncho. The top three genotypes that performed better than the standard and local checks for grain yield, as indicated in check should be considered together with RIL#17B when parental sources for better number of productive tillers per plant are needed. The mean value of panicle length was recorded as 42.46 cm with maximum of 39.23 cm and minimum of 46 cm for RIL#63A and RIL#113B, respectively. Maximum and minimum biomass yields were harvested from RIL#13A (6760.2 kg ha^{-1}) and Appendix Table 1, were RIL#10A, RIL#65A and RIL#3A with grain yield of 2962.7, 2842.2 and 2816 kg ha^{-1}, respectively.

Genetic variance, heritability and genetic advance

Estimated variance components, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) of the 13 studied traits of tef genotypes are presented in Table 2. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were regarded as low (<10%), moderate (10 to 20%) and high (>20%) as noted by Sivasubranian and Menon (1973), and Deshmukh et al. (1986). Therefore, high PCV was computed for yield per panicle and plant height. PCV and GCV values were computed as moderate for traits like biomass yield, grain yield, biomass production per day and grain production rate per day. Moderate GCV values of these characters suggest the possibility of improving these traits through selection. The phenotypic coefficient of variation was relatively greater than genotypic coefficient of variation for all these characters considered. This study is in agreement with the results reported by Jifar et al. (2015) and Jifar and Likyelesh (2013). In

Table 3. Minimum, maximum, mean values and variance components for 13 traits of tef genotypes.

Traits	Min	Max	Mean	σ^2_p	σ^2_g	GCV (%)	PCV (%)	H^2 (%)	GA	GAM (%)
DH	54	64.33	59.87	6.58	5.38	3.87	4.28	81.84	4.32	7.22
DM	101.67	117.67	108.1	25.93	24.92	4.62	4.71	96.14	10.09	9.34
PH	1.17	2.07	1.48	0.11	0.03	11.49	22.29	23.52	0.16	10.82
PL	98.7	118.33	108.23	28.51	21.32	4.27	4.93	74.78	8.24	7.61
NT	39.23	46	42.46	5.78	2.00	3.32	5.65	34.63	1.71	4.04
PW	11.03	16.7	13.71	0.03	0.01	7.3	12.6	31.85	0.11	8.29
YPP	5.1	9.47	7.42	0.04	0.01	13.51	27.03	25.00	0.10	13.94
BY	6760.2	11476.8	8393.5	1582078.57	1423392.47	14.21	14.99	89.97	2334.57	27.81
GY	1867.3	2962.7	2375.4	82318.48	66893.13	10.89	12.08	81.26	480.96	20.25
HI	22.9	32	28.47	4.47	2.89	5.97	7.41	64.62	2.81	9.88
LI	72.33	93	85.72	25.76	19.33	5.13	5.92	75.06	7.86	9.17
BPR	61.54	109.6	77.81	146.32	131.99	14.77	15.55	90.21	22.52	28.94
GYPD	16.87	28.3	22.03	8.49	7.07	12.07	13.16	83.28	4.98	22.62

DH = Days to heading, DM = days to maturity, PH = plant height (cm), PL = panicle length (cm), NT = number of productive tillers per plant, PW = panicle weight per plant per plant (g),YPP = yield panicle^{-1}(g) , TKW = thousand kernel weight (g), BY = biomass yield(kg ha^{-1}), GY = grain yield (kg ha^{-1}), HI = harvest index (%), LI = lodging index (%), BPR = biomass production rate (kg ha^{-1} day^{-1}), GYPG = grain yield production rate per day (kg ha^{-1} day^{-1}), σ^2_g = genotypic variance, σ^2_p = phenotypic variance PCV= phenotypic coeficient of varience (%), GCV = genotypic coeficent of varience (%), H^2 = broad sense heritability (%), GA = genetic advance, GAM = genetic advance as as percent of mean (%) and RILs = recombinant inbred lines.

contrast to this, Chanyalew (2010) reported high GCV than PCV for biomass yield, panicle seed yield and harvest index. The magnitude of the difference between PCV and GCV in this study was low for number of tillers, days to maturity, panicle length, biomass yield, lodging index and biomass production rate per day. This showed that the environmental effects on genetic expression of these traits were low and selection based on the phenotype or genotypes would result in genetic improvement which is eminent. This is in agreement with the report by Ayalew et al. (2012) for days to maturity and harvest index. Both GCV and PCV values were moderate for plant height, panicle yield, grain yield, biomass production rate per day and grain yield production rate per day. High PCV was noted for plant height and yield per panicle, while moderate PCV but low GCV values were computed for panicle weight. Both PCV and GCV values were computed as low for days to heading, days to maturity, panicle length, lodging index, harvest index and number of tillers. This is in line with the studies reported of Admas and Belay (2011), Debebe et al. (2012) and Jifar and Gugssa (2013).

The magnitude of differences between PCV and GCV for characters like plant height and yield per panicle were relatively high. This implies greater effects of environmental factors for the phenotypic expression of these characters. This may make it difficult to improve the characters by selecting high performing genotypes. This result is in close agreement with the findings of Jifar and Gugssa (2011) who reported relatively high PCV than GCV for plant height. In contrast, low PCV and GCV values were computed for days to heading, days to maturity, number of tillers, harvest index and lodging index.

Genotypic coefficient of variation provides information on the genetic variability present in various quantitative traits, but it is not possible to determine the extent of the variation that was heritable only from the genotypic coefficient of variation. Genetic coefficient of variation together with heritability would give clear estimate of the amount of advance to be expected from selection, Burton and De Vane (1953). According to Singh (2001), if very high or high, for example 80% or more heritability is accompanied by high genetic advance of a character, selection for such characters could be fairly feasible. This could be because of close correspondence between the genotype and the phenotype due to the relative small contribution of the environment to the phenotype. But, for characters with low heritability, for example 40% or less, selection may be considerably difficult due to the masking effect of the environment.

From the results presented in Table 2, very high estimate of heritability values were estimated for days to maturity, biomass production rate per day, biomass yield, grain yield production rate per day, days to heading (81.84%) and grain yield. This result suggested that selection of these traits could be fairly easy and advancement is possible using selection breeding This result is in line with that of Tefera et al. (2003b) and Jifar et al. (2015) who reported very high broad sense heritability estimates for days to maturity (85.59%), panicle length (96.07%) and days to heading (96.98%) in tef genotypes. On the other hand, medium heritability estimates were noted for harvest index, lodging index

and panicle length. Similar results were previously reported in tef for harvest index (78.2%), panicle length (74.78%) and lodging index (74%), by Jifar et al. (2013), Chanyalew (2010), and Ayalew et al. (2012), respectively.

Low heritability estimates were recorded for plant height, number of tillers, panicle weight and yield per panicle (Table 2) such low values indicated that improvement could be difficult for these characters through selection. Similar results showed low heritability for panicle weight and plant height as reported by Debebe et al. (2012) and for number of tillers by Chanyalew (2010).

Genetic advance as percent of mean ranged from 4.04% for number of tillers to 28.94% for biomass production rate per day. Johnson et al. (1955) classified genetic advance as percent of mean as low (<10%), moderate (10-20%) and high (>20%). Based on this classification, as presented in Table 2, traits like biomass yield, grain yield, biomass production rate per day and grain yield production rate per day recorded high genetic advance as percent of mean, while moderate genetic advance as percent of mean was recorded for plant height and panicle yield. Genetic advance under selection refers to progress in selected genotypes as compared to the base population with a single cycle of selection at a given selection intensity (Singh, 2001). Therefore, the results suggested that selecting the top 5% of the genotypes could result in genetic advance values of 4.04 to 28.94%.

Genetic advance values were low (<10%) for days to heading, days to maturity, panicle length, number of tillers, panicle weight, harvest index and lodging index (Table 2). This implies that advancement of traits in genotypic value for the new population as compared to the base population under one cycle of selection is <10% at 5% selection intensity. Similar work was reported by Jifar et al. (2013) who indicated that the genetic advance was low (<10%) for traits like days to heading (6.05%), days to maturity (0.80%), panicle length (5.18%) and lodging index (4.86%).

According to Johnson et al. (1955a), high heritability together with high genetic advances are more useful than heritability alone, implying the role of additive genes in the expression of the traits and thus it could be very effective in improvement and predicting the resultant effect on selecting the best individuals. In this study, high heritability together with high genetic advance values as percentage of the mean were observed for biomass yield, grain yield, biomass production rate per day and grain yield production rate per day. Hence, selection for such traits is likely to be effective. Similar results of high genetic advance show estimates of 39.1 and 68.6% in tef for grain yield by Jifar et al. (2015) and Admas and Belay (2011), respectively.

A relatively low heritability with low genetic advance were observed for harvest index, panicle weight and number of tillers. The low heritability of traits may be due to the presence of non-additive type of gene action (Ali et al., 2009).

Conclusion

The present study showed that there is a wide range of variability in the studied genotypes for most of the traits studied. Hence, progress could be achieved in seed yield through selection in tef crop.

ABBREVIATIONS

DH, Days to heading; **DM,** days to maturity; **PH,** plant height (cm); **PL,** panicle length (cm); **NT,** number of productive tillers per plant; **PW,** panicle weight per plant per plant (g); **YPP,** yield panicle^{-1}(g); **TKW,** thousand kernel weight (g); **BY,** biomass yield (kg ha^{-1}); **GY,** grain yield (kg ha^{-1}); **HI,** harvest index (%); **LI,** lodging index (%); **BPR,** biomass production rate (kg ha^{-1} day^{-1}); **GYPG,** grain yield production rate per day (kg ha^{-1} day^{-1}); σ^2_g, genotypic variance; σ^2_p, phenotypic variance; **PCV,** phenotypic coeficient of varience (%); **GCV,** genotypic coeficent of varience (%); H^2, broad sense heritability (%); **GA,** genetic advance, **GAM,** genetic advance as percent of mean (%); **RILs,** recombinant inbred lines.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

Admas S, Belay G (2011). Drought-resistance traits variability in Eragrostis tef X Eragrostis pilosa recombinant inbred lines. Afr. J. Agric. Res. 6:3755-3761.

Alaunyte I, Stojceska V, Plunkett A, Ainsworth P, Derbyshire E (2012). Improving the quality of nutrient-rich tef (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough bread making, J. Cereal Sci. 55:22-30.

Ali MA, Nawab NN, Abbas A, Zulkiffal M, Sajjad M (2009). Evaluation of selection criteria in Cicer arietinum L. using correlation coefficients and path analysis. Australian J. Crop Sci. 3:65-70.

Allard RW (1960). Fundamentals of Plant Genetics and Breeding. John Willey & Sons, New York. England.

Assefa K, Tefera H, Merker A, Kefyalew T, Hundera F (2001a). Variability, heritability and genetic advance in pheno-morphic and agronomic traits of tef [Eragrostis tef (Zucc.) Trotter] germplasm from eight regions of Ethiopia. Hereditas 134:103-113.

Assefa K, Yu JK, Zeid M, Belay G, Tefera H, Sorrells MS (2011). Breeding tef [Eragrostis tef (Zucc.) trotter]: conventional and molecular approaches. Plant Breed. 130:1-9.

Ayalew H, Tsige G, Tadesse D, Landuber W (2011). Multivariate diversity, heritability and genetic advance in tef landraces in Ethiopia. Afr. Crop Sci. J. 19:201-212.

Board on Science and Technology for International Development, National Research Council (BoSTID) (1996). Lost Crops of Africa: 1: Grains (Washington, DC: National Academy Press).

Burton GW, De Vane EH (1953). Estimation of heritability in Tall

Festuca (Festuca arundinacea) from replicated clonal material. Agron.J. 45:478-481.
Cannarozzi G, Plaza WS, Esfeld K, Larti S, Wilson YS, Dejene G, De Castro E, Chanyalew S, Blosch R, Farinelli L, Lyons E, Schneider M, Falquet L, Kuhlemeier C, Kebebew A, Zerihun T (2014). Genome and transcriptome sequencing identifies breeding targets in the orphan crop tef (Eragrostis tef). BMC Genomics 15.
Central Statistical Agency (CSA) (2014). Agricultural sample survey for 2014. CSA Addis Ababa, Ethiopia. Volume III.
Chanyalew S (2010). Genetic analyses of agronomic traits of tef (Eragrostis tef) genotypes. Res. J. Agric. Biol. Sci. 6:912-916.
Debebe A, Singh H, Tefera H (2013). Interrelationship and path coefficient analysis of F4 progenies of tef [Eragrostis tef (Zucc.) Trotter]. Pakistan J. Biol. Sci. 4:225-228.
Debebe A, Singh H, Tefera H (2014). Interrelationship and path coefficient of yield components in f4 F4 progenies of tef [Eragrostis tef (Zucc.) Trotter]. Pakistan J. Biol. Sci. 17(1):92-97.
Deshmukh SN, Basu MS, Reddy PS (1986). Genetic variability, character association and path coefficient analysis of quantitative traits in Viginia bunch varieties of ground nut. Indian J. Agric. Sci. 56:515-518.
Ebba T (1975). tef (Eragrostis tef) cultivars: morphology and classification, Part II. Debre Zeit Agricultural Research Station. Bulletin Number 66, Addis Ababa University, Dire Dawa, Ethiopia.
Gomez AK, Gomez AA (1984). Statistical Procedures for Agricultural Research.John Willey and Sons Inc., New York 657 p.
Jifar H, Assefa K, Tadele Z (2015). Grain yield variation and association of major traits in brown-seeded genotypes of tef [Eragrostis tef (Zucc.)Trotter]. Agric. Food Security 4:7.
Jifar H, Endashaw B, Kebebew A (2011). Genetic variability in released tef [Eragrostis tef (Zucc.) Trotter] varieties of Ethiopia. Crop Science Society of Ethiopia (CSSE). Sebil 13. Proceedings of the Thirteenth Biennial Conferences, 31st Dec. 2008 to 2nd Jan. 2009. Addis Ababa, Ethiopia.
Jifar H, Gugssa L (2013).Variation in Major Traits of Gynogenically derived tef [Eragrostis tef (Zucc.)Trotter] lines Evaluated in the Central highlands of Ethiopia. Ethiopian J. Appl. Sci. Technol. (4):50-64.
Johnson HW, Robinson HF, Comstock RF (1955b). Genotypic and phenotypic correlation in Soya bean and their implication in selection. Agron. J. 47:477-483.
Ketema S (1993). tef (Eragrostis tef): Breeding, Agronomy, Genetic Resources, Utilization, and Role in Ethiopian Agriculture. Institute of Agricultural Research, Addis Abeba, Ethiopia.
Ketema S (1997). Tef Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.
Khan SA (2013). Genetic Variability and Heritability Estimates in F2 wheat Genotypes. Int. J. Agric. Crop Sci. 5(9):983-986.
Mengesha M, Picket RC, Davis RI (1965). Genetic Variability and inter-relationship of characters in tef [Eragrostis tef (Zucc). Trotter]. Crop Sci. 5:155-157.
Plaza SW, Cannarozzi G, Zerihun T (2013). Genetic and phenotypic diversity in selected genotypes of tef [Eragrostis tef (Zucc.) Trotter. Afr. J. Agric. Res. 8:1041-1049.
SAS Institute Inc. (2004). SAS/STAT. 9.1.3 User's Guide. Cary, NC: SAS Institute Inc, USA. And Sons, Inc., New York.
Singh BD (2001). Plant Breeding principles and methods. Kalyani publishers, New Delhi 896 p.
Sivasubranian S, Menon M (1973). Hetrosis and inbreeding depression in rice. Madras Agric. J. 60:1139-1144.
Spaenij-Dekking L, Kooy-Winkelaar Y, Koning F (2005). The ethiopian cereal tef in celiac disease. New England J. Med. 353:1748-1749.
Tefera H, Assefa K, Belay G (2003a). Evaluation of interspecific recombinant inbred lines of Eragrostis tef x E .pilosa. J. Genet. Breed. 57:21-30.
Tefera H, Assefa K, Hundera F, Kefyalew T, Teferra T (2003b). Heritability and genetic advance in recombinant inbred lines of tef (Eragrostis tef). Euphytica 131:91-96.
Vavilov I (1951). The origin, variation, immunity and breeding of cultivated plants. Translated from the Russian by K. Starrchester Ronald Press, New York.
Watson L, Dallawitz MJ (1992). The Grass Genus of the World. CAB International, Wallingford, Oxon, UK.

Appendix 1. Mean yield and yield components performance values of 14 traits of 37 tef genotypes tested.

Genotypes	DH	DM	PH	PL	NT	PW	PY	BYLD	GYLD	HI	LI	BPR	GYPD	TKW
Kora	58.67	116.33	112.8	39.83	1.63	11.9	6.1	10866.8	2711.8	24.9	92	93.4	23.31	0.37
Local	58	116.67	106.2	43.13	2.07	14.9	7.55	7834.2	2203.4	28.14	91.67	67.17	18.89	0.33
Quncho	57	115.33	114	43.1	1.3	14.2	8.17	8632.8	2302	26.66	89.67	84.38	22.49	0.47
RIL#10A	57	104.67	105.47	40.77	1.2	13.6	7.57	11476.8	2962.7	25.8	93	109.6	28.3	0.33
RIL#110A	63.33	105	105.87	42.87	1.7	13.17	7.27	8247.7	2340.7	28.36	84.33	78.57	22.29	0.33
RIL#113B	60	104	103.8	39.23	1.43	12.97	7.57	8032.8	2380	29.6	81.67	77.25	22.88	0.33
RIL#11B	63	115.67	112.23	39.9	1.37	11.5	5.1	7284	1952.8	26.8	82.67	62.9	16.88	0.33
RIL#11C	62.33	116	103.67	42.57	1.43	13.4	7.23	7561.8	2415.8	32.01	82.33	65.17	20.83	0.4
RIL#11D	61.67	116	110.93	42.23	1.33	13.9	6.4	8139	2353.3	28.9	90	70.16	20.28	0.37
RIL#11E	61.67	110.67	114.27	42.1	1.17	14.8	8.27	11449.3	2627.2	22.9	90	103.45	23.73	0.33
RIL#121A	63.33	108.67	110.43	43.5	1.23	13.4	8.1	8283.8	2367.2	28.55	83.33	76.24	21.78	0.37
RIL#124A	57	113c	111	43.07	1.8	11.03	5.3	8449.8	2256.2	26.8	85.67	74.8	19.96	0.27
RIL#124B	59.67	111.33	105.13	44.23	1.5	12.97	6.77	7619.5	2103.8	27.6	83	68.44	18.89	0.37
RIL#13A	59	107	102	40.17	1.6	14.3	7.8	6760.2	2118.2	31.35	92	63.19	19.79	0.33
RIL#16A	64.33	107.33	110.1	40.5	1.37	12.67	7	8647.5	2636	30.5	83.33	80.57	24.56	0.3
RIL#17A	58.67	111	104.37	41.4	1.97	13.47	7.5	8346.2	2589.2	31.02	90	75.2	23.33	0.37
RIL#17B	60	115	98.7	40.13	1.17	13.8	7.1	7089.8	2015.3	28.38	72.33	61.6	17.52	0.3
RIL#19A	58.33	105	107.33	44.77	1.27	16.13	7.3	9264	2701.3	29.15	86	88.23	25.73	0.33
RIL#19B	60.33	107	102.27	42.07	1.3	14.33	8.13	7379.3	2222	30.1	89	68.9l	20.76	0.33
RIL#28B	54	110.33	112.7	43.37	1.4	13.1	6.47	7530.3	2306.8	30.6	86	68.27	20.9	0.33
RIL#3A	58	105.33	118.3	45.07	1.47	14.5	8.4	9391.3	2816	29.9	90.33	89.17	26.74	0.27
RIL#3C	62	107	110.13	42.63	1.4	14.27	8.27	9531.8	2642.3	27.8	89	89.07	24.69	0.27
RIL#44A	59.67	101.6	108.43	39.97	1.67	12.9	7.37	8353.7	2601	31.16	82.67	82.14	25.58	0.27
RIL#44B	60	103	118.33	43.03	1.5	13.4	8.07	7720	2236.2	28.9	85	74.9	21.7	0.37
RIL#45B	61.67	116.67	104.23	41.2	1.27	12.33	6.03	6810	1867.3	27.42	77.33	61.5	16.87	0.27
RIL#46C	62	117.67	115.37	45.97	1.47	12.33	6.3	8373.8	2365.6	28.32	80.33	71.16	20.1	0.3
RIL#48A	59	102.67	103.3	44.47	1.5	14.6	9.4	7224.3	2053.8	28.43	86	70.39	20.01	0.43
RIL#50B	56l	102	103.5	41.1	1.53	11.9	6.67	7150.8	2094	29.3	82.33	70.1	20.53	0.4
RIL#57B	60.33	107	103.27	40.67	1.3	12.87	7.33	8718.9	2532.3	29.05	82.67	81.49	23.67	0.33
RIL#5B	58.33	102	108.27	44.4	1.9	16.6	9.47	7749.3	2217.5	28.58	92	75.9	21.73	0.3
RIL#63A	63	102	107.6	46	1.47	13	6.47	7446.8	2140	28.74	83.33	73.03	20.98	0.33
RIL#65A	61	101.33	112.05	44.8	1.67	15.5	8.6	10347	2842.2	27.6	90.33	93.8	25.76	0.37
RIL#68A	63.67	105	102.7	41.3	1.77	13.13	7.2	8644.8	2559.7	29.6	90.67	82.4	24.38	0.4
RIL#70A	57.67	109.67	106.7	42.13	1.8	16.7	8.87	8359	2356.3	28.23	83.67	76.23	21.48	0.37
RIL#74C	58	102	110.9	43.3	1.27	13.8	7.47	9401.5	2531.3	26.9	82.67	92.19	24.8	0.33
RIL#75B	60	104.67	114.2	44	1.37	15.6	8.33	9554.3	2485.3	26.02	83.67	91.28	23.75	0.33

Appendix 1. Contd.

RIL#8B	57.67	116	103.67	41.87	1.2	14.2	7.7	6887.5	1984.5	28.78	81.67	66.24	19.1	0.3
Mean	59.87	108.1	1.48	108.23	42.46	13.71	7.42	8393.5	2375.4	28.47	85.72	77.81	22.03	0.33
CV	1.83	0.93	19.54	2.48	4.58	10.93	12.42	21.07	4.75	5.23	4.42	2.96	4.87	21.067

DH = Days to heading, DM = days to maturity, PH = plant height (cm), PL = panicle length (cm), NT = number of productive tillers per plant, PW = panicle weight, PY = panicle yield (g^{-1}) , TKW = thousand kernel weight (g), BY = biomass yield(kg ha^{-1}), GY = grain yield (kg ha^{-1}), HI = harvest index (%), LI = lodging index (%), BPR = biomass production rate (kg ha^{-1} day^{-1}) and GYPG = grain yield production rate per day (kg ha^{-1} day^{-1})

Vol. 13(49), pp. 2798-2803, 6 December, 2018
DOI: 10.5897/AJAR2018.13506
Article Number: 8A7968D59496
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Economic and operational analysis of tomato mechanized harvesting systems for industrial processing

Tulio de Almeida Machado*, Haroldo Carlos Ferandes, Fábio Lucio Santos, Nerílson Terra Santos and Clarice Aparecida Megguer

Instituto Federal Goiano - Campus Morrinhos, Goiás State, Brazil.

Received 31 August, 2018; Accepted 29 October, 2018

The determination of the cost of agricultural production is an important tool for controlling and managing productive activities and generating information to support decision-making. The objective of this work was to make technical and economic evaluations of three mechanized tomato harvesting systems for industrial processing through the study of times, movements and the determination of operational costs. The research was conducted during the year 2018 in the municipality of Morrinhos-GO. The productive and unproductive times were collected, and subsequently, an economic analysis of each one as well as the calculation of the internal rate of return according to the useful life of each system were performed. After the data collection and analysis, it was concluded that the productive and unproductive times were similar for the evaluated systems. Only the system formed by the harvester, tractor, hauling and bucket was different from the others in relation to the values in US$ h^{-1} and US$ ha^{-1}. From the fourth year, the internal rate of return was positive for all systems evaluated.

Key words: Costs of production, times and motion study, *Solanum lycopersicum.*

INTRODUCTION

Brazil is the 5th largest producer of tomato for industrial processing in South America and she leads the production, being the largest consumer market for its industrialized derivatives. Among the Brazilian states with the highest production of this variety, the state of Goiás stands out, with a transplanted area of 12,670 ha and an average yield of 75,000 kg ha^{-1} (Camargo et al., 2016).

Mechanization has been developing more and more in the different stages of the productive cycle, making possible the substitution of manual labor through the mechanization of crops (Fernandes et al., 2012).

Harvest aid machines can be a valuable alternative for improving labor conditions in the field and increasing harvest yield (Sarig, 2012; Elkins, 2012). Mechanization that replaces hired labor focuses on replacing labor in high-valued crops such as fruits and vegetables. At the beginning of the 20th century, this replacement led to debates about labor-push or labor-pull, where agricultural labor was used in the growing industrial sector (Schmitz and Moss, 2015).

Mechanized harvest of industrial tomato in Brazil has shown greater technical/economic reliability due to better

*Corresponding author. E-mail: machado.tulio@gmail.com.

cost-benefit ratio, making it attractive for most producers who practice it (Machado et al., 2014).

In this context, the mechanized harvesting of industrial tomatoes becomes important, because if the losses resulting from this operation reduces, there will be an increase in productivity per area; consequently, reflecting in the highest total production in the country (Casa and Evangelista, 2009).

Thus, the maximum utilization of machine functions with the improvement of harvesting techniques; resulting in the maximization of the use of the functions of the factors of production and increased of yield continuously (Pereira et al., 2015).

Regarding the costs of harvesting, the first harvester reduced harvest costs to 33% of total costs. After the electronic sorter was introduced in 1975, harvester costs dropped to 16% of total costs by 1979. Harvest costs have slowly declined since then (Huffman, 2010).

The systematic monitoring of the performance of agricultural machinery and calculations of their operating costs are fundamental factors for rational use. In this way, the operational performance of a machine refers to a complex set of information, which define their attributes, when operations are performed under certain conditions (Piacetini et al., 2012).

Knowledge of operational performance of an agricultural machine has become a growing concern and of utmost importance, because with the advent of mechanization the production costs were directly influenced by the efficiency of the machine in the field (Simões and Silva, 2012).

In this context, the objective of this work was to make technical and economic evaluations of three mechanized tomato harvesting systems for industrial processing; using combinations of equipment formed by the harvester, truck, hauling, bucket, and tractor.

MATERIALS AND METHODS

The study was conducted at Fazenda Santa Rosa, located in the municipality of Morrinhos, Goiás, with the longitude and the latitude of 17°44'31.7"S and 49°03'12.6"W, respectively and an average altitude of 770 m. The research was conducted in the year 2018. The experimental area was restricted to 300 ha for each evaluated system with slightly wavy relief (10%). At harvest time, the predominant soil of the type Dark Red Latosol was with the average water content of 20% (Embrapa, 2013).

The plant material used in this work was tomato cultivar Heinz 9553, which was transplanted in the area using the no-tillage system, with the harvesting process being fulfill approximately 125 days after culture introduction.

The soil corrections and irrigation for the crop were implemented according to the recommendations used for commercial cultivation. The material was transplanted in double rows and at the end of the harvest; it obtained an average yield of 105 tons ha^{-1}.

The equipment used were a self-propelled harvester of the brand Guaresi, model G-89/93 MS 40", with FIAT-Iveco engine of 128.7 kW, with floating collection platform; a truck of the Volkswagen brand, model 31.330, with Cummins ISL engine of 242.7 kW of power and traction 6×4 with body to transport rollon/off buckets of 40 m^3; a hauling with 2-axle double wheels with chassis and shock absorber itself of the Imavi brand; and a tractor of the John Deere brand, model 6.130J, with 95.6 kW of nominal power in the engine. Each harvester evaluated, harvested a double row at a time.

To measure the times, a digital chronometer and an extra chronometer were used for case of failures. The collected measures were applied in the scale of seconds, being composed by the time spent in the conduct of harvesting operations, as well as stops of the maneuvers and the displacements, during an eight-hour day's work. For the measurement of the operational velocity, each experimental plot had an area of 60 m^2 (50 m × 1.2 m) where the harvesters already entered the plot in full working regime.

The times measured were classified as productive and unproductive. The productive times were spent during the action of the machined sets in the field, being determined from the displacements to the execution of harvesting operations.

For the unproductive times, it was considered: auxiliary time (composed of the cleaning time of the harvester and the time for coupling and uncoupling of the hauling), time for maneuvers (sum of maneuver times of each harvesting system) and time for repair and maintenance. The productive and unproductive times of three harvesting systems, that were treated as experimental units and formed by the equipment: system 1, a harvester, a truck, a hauling and two buckets; system 2, a harvester, a tractor, a hauling and a bucket; and system 3, a harvester, a truck and a bucket.

A randomized complete block design was used where 10 repetitions were considered for each time measured in each harvesting system, and the mean of the observed times was used for the determination of field yields and effective field capacity of each harvesting system in the evaluated areas.

The mechanical availability, according to Simões et al. (2010), is defined as the percentage of working time, associated with the machine mechanically able to develop its operations, which comprises disregarding the time spent to perform repairs or maintenance (Equation 1).

$$D_m = \left(\frac{T_{pro}}{T_{pro} + T_{rep}}\right) 100 \quad (1)$$

where D_m: degree of mechanical availability, %; T_{pro}: productive time, h; and T_{rep}: interruption time for repairs or maintenance, h.

The efficiency of use presents equivalence in relation to the hours used and the total hours; consequently, it comes from the unproductive time of the agricultural machine (Equation 2).

$$E_u = \left(\frac{T_{pro} + T_{aux}}{T_{pro} + T_{imp}}\right) 100 \quad (2)$$

where E_u: utilization efficiency,%; T_{pro}: productive time, h; T_{aux}: auxiliary time, h; and T_{imp}: unproductive time, h.

To determine the percentage of time effectively worked, the operational efficiency was calculated according to the methodology proposed by Leite et al. (2012), as presented in Equation 3.

$$E_o = \left(\frac{T_{pro}}{T_{pro} + T_{imp}}\right) 100 \quad (3)$$

where E_o: operating efficiency, %; T_{pro}: productive time, h; and T_{imp}: unproductive time, h.

After the data acquisition, a variance analysis was performed for these values, and subsequently subjected to the Tukey test at 5% probability.

The initial values of the acquisition of the machines and

Table 1. Initial values of acquisition, useful life and working hours per year of machines and implements used in tomato harvesting for industrial processing.

Used equipment	Initial value (US$)	Hours worked/year (h)	Useful life (years)
Truck	84,541.06	873	10
Hauling	16,908.21	873	10
Bucket	2,898.55	873	10
Tractor of 95.6 kW	47,111.44	873	10
Harvester	314,009.66	873	8

Table 2. Mechanical availability, utilization efficiency and operational efficiency of the evaluated systems.

System	Mechanical availability (%)	Utilization efficiency (%)	Operational efficiency (%)
System 1	89.73[a]	87.51[a]	85.91[a]
System 2	90.29[a]	88.39[a]	87.28[a]
System 3	89.40[a]	87.62[a]	85.95[a]

Averages followed by the same letter in column, do not differ statistically among themselves, by Tukey test at 5% probability.

implements were acquired through consultations in resales of the region and are shown in Table 1, where the descriptions of the useful life and the number of hours worked per year are also arranged. Initial values were considered after consulting the machine dealers in the region. The useful life values were the same as those obtained by the CONAB methodology (2010).

After determining the hourly cost of each machine set, the operating costs were expressed in American commercial dollars, official of the Central Bank of Brazil (PTAX 800), at the selling price, per hour of work (US$ h^{-1}). It was considered as exchange rate the price of foreign currency, measured in units and fractions of the national currency, in the amount of R$ 4,14 (30/08/2018).

Operating costs were estimated using the same methodology proposed by Machado et al. (2017). Operating costs were composed by fixed costs and variable costs. Fixed costs composed of depreciation, interest on invested capital and expenses with shelter, insurance and taxes. The variable costs composed of labor, fuels, lubricants, and repair and maintenance costs.

Subsequently, the operational costs in productive and unproductive times of each system were compared by the Tukey test at 5% probability. Statistical analyses were performed using the Minitab 17.0 software.

The annual revenue of each system was calculated considering the total production in each area by the value of the ton of tomato harvested (harvester) or by the value of the ton of tomato transported to industry (transport). The values were separated according to each evaluated system. The average productivity in the area was 100 t ha^{-1}, the value of the ton harvested of R$ 23.00 and the value of the transported ton of R$ 25.00. These values are the values consulted in agroindustries and were practiced in the region during the harvest period.

In determining the cost of production, only the fraction of the total time was considered, during which the harvesting system was programmed to perform productive work, that is, the time actually spent at work.

The annual cost of each system was calculated from the sum of operating costs and the acquisition value of each equipment. For the subsequent years, only the operational cost was considered, and in the last year of the useful life, the residual value of each equipment was added to the operational cost.

To evaluate the attractiveness of the evaluated systems, the Internal Rate of Return (IRR) was calculated, which represents the real profitability of the investment, and for that reason is considered the internal rate of the enterprise. According to Lanna and Reis (2012), it was obtained with the support of Equation 4, expressed as a percentage.

$$\sum_{j=1}^{n} R_j(1+TIR)^{-j} - \sum_{j=1}^{n} C_j(1+TIR)^{-j} = 0$$

where IRR: internal rate of return, %; R_j: revenue from the period of time j considered, US$; C_j: costs from the period of time j considered, US$; and N: duration of the project, years.

For the comparison of IRR, the minimum rate of attractiveness of the investment used for the present study was the selic rate that on 30/08/2018 was 7% per year.

RESULTS AND DISCUSSION

The mechanical availability, efficiency of use and operational efficiency were studied using analysis of variance. The means of the variables evaluated did not differ among the harvesting systems used (Table 2). The average speed of the harvesters during the operation was 3.93 km h^{-1}.

It can be observed that the mechanical availability in the different harvest systems was around 89%, that can be explained by the greater proportional time spent to perform corrective maintenance, predicted in the unproductive times, during the operation that consequently generated a decrease in efficiency of use, justified mainly for the loss or impediment of work due to unproductive time. Time spent with repair and maintenance were the same in all three systems, because as there is dependence between the harvester and the transport system, when the equipment is stopped the operation of the other is compromised.

Table 3. Productive, auxiliary, maneuvers and repairs time for the different evaluated systems.

System	Productive time (h)	Auxiliary Time(h)	Maneuvers Time(h)	Repairs time(h)	Unproductive time (h)
System 1	514.25 a	9.55 a	15.88 a	58.88 a	84.31 a
System 2	524.45 a	6.67 a	13.32 a	56.42 a	76.41 a
System 3	519.35 a	10.10 a	13.49 a	61.32 a	84.91 a

Using Tukey test at 5% probability, averages followed by the same letter in column do not differ statistically among themselves.

Table 4. Production costs in US$ h^{-1} (%) for the evaluated systems.

Parameter	System 1 (%)	System 2 (%)	System 3 (%)
Depreciation	34.32 (44.26)	33.34 (48.17)	32.52 (44.77)
Interest	7.77 (10.02)	7.46 (10.78)	7.20 (9.91)
Shelter, insurance and taxes	2.69 (3.47)	1.76 (2.54)	2.58 (3.55)
Fuel	18.92 (24.40)	14.13 (20.41)	16.86 (23.21)
Lubricants	3.42 (4.41)	2.87 (4.15)	3.37 (4.64)
Repair and maintenance	8.70 (11.22)	7.89 (11.40)	8.38 (11.54)
Labor	1.72 (2.22)	1.77 (2.55)	1.72 (2.38)
Total	77.56 (100)	69.22 (100)	72.64 (100)

As there is no similar research in tomato harvesting, the comparison with other crops is necessary. In this context, the objective is to evaluate technically and economically the performance of a harvester at harvest of eucalyptus in forest of first cut. Simões et al. (2010) observed all the experimental plots, for an average mechanical availability of 92.04% that resulted in an average operating efficiency of 91.53% by effective working hours. These values show that the operational efficiency in the eucalyptus harvest in the situation described by the authors is greater than that found in the present study.

The values of operational efficiency were around 86% and are due to less time spent with unproductive times, characterized by a longer productive time spent during the harvesting operation. Evaluating self-propelled harvesters in irrigated rice harvesting, Araldi et al. (2013), concluded that the average operating efficiency in different types of systematization of the soil was 65% with minimum values of 50.8% and maximum values of 77.6%. This shows that the values found in the present work show high efficiency during harvesting in the three evaluated systems.

Table 3 shows the productive and unproductive times for each harvesting system, where the values did not differ from each other. The values were obtained for the harvest of 300 ha in each evaluated system.

In relation to the highest value of productive time, the results are explained by the fact that the harvesting operation was performed at a slower speed and with few stops during the activity; consequently, there was less unproductive time spent with stops, maintenance and maneuvering.

The values of auxiliary times of the machines did not influence the systems, characterized as fast operations, which adjusts well to the harvesting systems that use it. These systems presented a greater facility for performing the maneuvers in relation to system 1. However, this condition did not result in differences in relation to the harvesting system regarding the time spent on this issue.

Harvesting systems presented the same behavior, where the productive times were greater than the unproductive times, which is explained by the values of mechanical availability, efficiency of use and operational efficiency. The values presented indicate a longer time of mechanized sets in operation during the harvesting process.

In Table 4, hourly production costs of each system were separated in fixed costs and variable costs.

The total hourly cost of system 1 was the highest value among the analyzed systems. The sum of fixed and variable costs made this operation cost 77.56 US$ h^{-1}. The fact can be explained by a greater initial value for the truck used in the execution of the operation. In reverse of system 2, the tractor had a lower acquisition cost, which reduced the operating costs. In the three systems analyzed, the highest value for fixed costs was found for the depreciation and for variable costs; while the highest value was spent on fuel.

In this same context and corroborating with the present work, Cunha et al. (2015) evaluated different types of coffee harvesting and concluded that the factors of depreciation, fuel, repairs and maintenance were the elements of the costs that had greater participation in the

Table 5. Costs in productive, unproductive and total times per hectare in each system.

System	Productive (US$ ha^{-1})	Unproductive (US$ ha^{-1})	Total (US$ ha^{-1})
System 1	$108.67 (87.17%)[a]	$15.65 (12,83%)[a]	$124.32 (100%)[a]
System 2	$98.92 (86.90%)[b]	$14.91 (13.10%)[b]	$113.83 (100%)[b]
System 3	$102.80 (86.77%)[a]	$15.67 (13.23%)[a]	$118.47 (100%)[a]

Using Tukey test at 5% probability, averages followed by the same letter in column do not differ statistically among themselves.

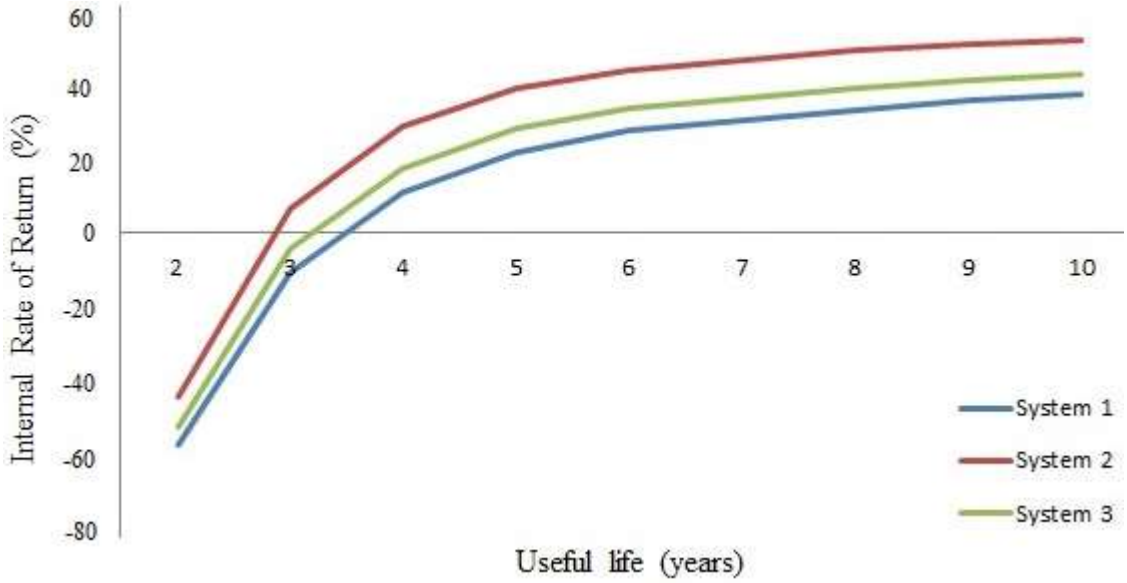

Figure 1. Internal rate of return for the three systems evaluated.

operating costs of the studied mechanized systems.

Oliveira et al. (2009) who analyzed the forest harvest of a forwarder in the extraction of pine logs concluded that fixed costs accounted for 42.8% of total operating costs explained this behavior, and the depreciation obtained 34.1%, which was the factor that mostly influenced the result.

In this context, Simões et al. (2011) analyzed in a subsoiling operation implantation of a commercial forest that the fuel item is the main component among others, which composed of the operating cost of agricultural machinery, directly affecting the final costs of production.

Table 5 shows the results of costs for the realization of different harvesting systems per hectare, considering the costs associated with productive times and unproductive times.

System 2, in addition to differentiating itself from the other harvesting systems, was the one that presented the smallest difference between productive and unproductive times. The total cost of operation in system 2 was lower than systems 1 and 3, because of the lower value of acquisition for tractor, while the others used a truck. These results corroborate with Janini (2008), evaluating mechanized and semi-mechanized transplantation of sugarcane. Oliveira et al. (2009) and Santos et al. (2016) evaluated different forest harvesting systems and concluded that the greater the operational efficiency of a system, the lower the cost of your operation.

The IRR was calculated for different harvesting systems, as shown in Figure 1. In system 2 it was -43.07% in the second year. In the third year, the value started positively (8.97%), and it obtained increasing values until the end of the useful life (52.88%). This system obtained the highest initial IRR value because the acquisition value of the tractor is lower than the truck, and this directly influenced the result of the useful life.

System 1 obtained lower value at the end of the useful life of your equipment and it took longer time to obtain positive values over the years. The fourth year of use presented positive value, indicating that the system is paid only from that year. In the other years, until the end of the useful life, the value of IRR increased continuosly. At the end of its useful life, system 1 was paid and it generated a gain of 38.48% on services provided. System 2 obtained a positive IRR value in the third year, having a return to a shorter term.

In this context, from the detailing of the costs of production in the forest harvest, Santos et al. (2016) evaluated that the maximum value of the IRR on the investment of a harvester and a forwarder was obtained in the fifth year. This is useful for two evaluations, with depreciation up to the sixth year of useful life and with depreciation until the fourth year of useful life being the percentage of the order with 34 and 21%, respectively.

All the systems presented superior results in relation to attractiveness rate, considering the selic rate of 12.25% per year, and it demonstrated that the activity is profitable until the end of the useful life of the equipments studied.

Knowledge of economic values, which are part of the culture cycle of industrial tomato, are important to determine the amounts paid to producers. Therefore, new techniques are necessary to reduce production costs and to make the business more attractive within the agribusiness chain.

Conclusions

There was no difference between the factors of mechanical availability, efficiency of use and operational efficiency among the evaluated harvesting systems.

For all harvesting systems, the fixed costs were higher than the variable costs, for values in US$ h^{-1} and for the values in US$ ha^{-1}.

Only the system formed by the harvester, tractor, hauling and bucket (system 2) obtained a lower cost than the others in relation to the values in US$ h^{-1} and US$ ha^{-1}. System 1 presented higher values for costs per hectare when compared with others. System 2 obtained a positive value for Internal Rate of Return after the third year of harvest, while systems 1 and 3 had a positive value after the fourth year of the equipment's useful life.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Camargo MS, Brito Júnior JS, Molena LA (2016). Alta nos custos limita rentabilidade em 2016. Anunário hortifruti Brasil 163:14-17.

Casa J, Evangelista RM (2009). Influência das épocas de colheita na qualidade de tomate cultivado em sistemas alternativos. Semina: Ciências Agrárias 30 (1):1101-1107.

Companhia Nacional de Abastecimento (Conab) (2010). Custos de produção agrícola: a metodologia da Conab. Conab 60 p.

Cunha JPB, Silva FM, Andrade F, Machado TA, Batista FA (2015). Análise técnica e econômica de diferentes sistemas de transplantio de café (*Coffea arábica L.*). Coffee Science 10(3):289-297.

Embrapa – Empresa Brasileira de Pesquisa Agropecuária (2013). Sistema Brasileiro de Classificação de Solos. Embrapa 353 p.

Elkins RB (2012) A Historical timeline of research efforts to implement mechanized pear harvest in California. Acta Horticulturae 965(30):225-230.

Fernandes ALT, Partelli FL, Bonomo R, Golynski A (2012). A moderna cafeicultura dos cerrados brasileiros. Pesquisa Agropecuária Tropical 42(2):231-240.

Huffman WE (2010). The status of labor-saving mechanization in fruits and vegetables. Economics Working Papers (126):1-47.

Janini DA (2008). Análise operacional e econômica do sistema de plantio mecanizado de cana-de-açúcar (*Sacharum spp.*) STAB 26 (4):51-57.

Lanna GBM, Reis RP (2012). Influência da mecanização na colheita na viabilidade econômico-financeira da cafeicultura no sul de Minas Gerais. Coffee Science 7(2):110-121.

Leite ES, Fernandes HC, Guedes IL, Amaral EJ, Lacerda EG (2012). Utilização de guindaste na extração de madeira em região montanhosa. Revista Árvore 36(1):571-580.

Machado TA, Santos FL, Fernandes HC, Valente DSM, Cunha JPB (2017). Technical and economic analysis of industrial tomato transplanting system. Revista Espacios 38(13):07-16.

Machado TA, Coelho LM, Cunha, JPB, Santos FL (2014) Configuração acertada. Cultivar Máquinas 136(12):33-35.

Oliveira D, Lopes ES, Fiedler NC (2009). Avaliação técnica e econômica do Forwarder na extração de toras de pinus. Scientia Forestalis 37(84):525-533.

Pereira ALN, Lopes ES, Dias NA (2015). Análise técnica e de custo do Feller-Buncher e Skidder na colheita de Madeira em diferentes produtividades do povoamento. Ciência Florestal 25(4):981-989.

Piacetini L, Souza EGD, Opazo MAU, Nobrega LHP, Milan M (2012). Software para estimativa do custo operacional de máquinas agrícolas maqcontrol. Engenharia Agrícola 32(3):609-623.

Santos LN, Fernandes HC, Teixeira MM, Silva ML, Souza AP (2016). Avaliação econômica da colheita florestal mecanizada com Harvester e Forwarder. Revista Árvore 40(6):1077-1082.

Sarig Y (2012). Mechanical Harvesting of Fruit – Past Achievements, Current Status and Future Prospects. Acta Horticulturae 965(21):163-170.

Schmitz A, Moss CB (2015). Mechanized agriculture: Machine adoption, farm size, and labor displacement. AgBioForum 18(3):278-296.

Simões D, Iamonti IC, Fenner PT (2010). Avaliação técnica e econômica do corte de eucalipto com Feller-Buncher em diferentes condições operacionais. Ciência Florestal 20(4):649-656.

Simões D, Silva MR, Fenner PT (2011). Desempenho operacional e custos da operação de subsolagem em área de implantação de eucalipto. Bioscience Journal 27(5):692-700.

Simões D, Silva MR (2012). Desempenho operacional e custos de um trator na irrigação pós-plantio de eucalipto em campo. Revista Ceres 59(2):164-170.

Vol. 13(49), pp. 2804-2807, 6 December, 2018
DOI: 10.5897/AJAR2018.13414
Article Number: 487E8AF59498
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Priming of *Urochloa brizantha* cv. Xaraés seeds

Silvia Rahe Pereira[1], Ana Eliza da Silva Lima[2], Adriana Paula D'Agostini Contreiras-Rodrigues[3], Daiane Rodrigues de Oliveira[1] and Valdemir Antônio Laura[1,4,5*]

[1]Programa de Pós-graduação em Produção e Gestão Agroindustrial, Universidade Anhanguera-Uniderp, Campo Grande, Mato Grosso do Sul, Brasil.
[2]Fundação Educacional de Andradina, Andradina, São Paulo, Brasil.
[3]Universidade Tecnológica Federal do Paraná, Campus Pato Branco, Pato Branco, Paraná, Brasil.
[4]Embrapa Gado de Corte, Campo Grande, MS, Brasil.
[5]Programa de Pós-graduação em Biologia Vegetal, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil.

Received 24 July, 2018; Accepted 5 November, 2018

Priming is one of the several physiological methods used to increase the performance of seeds. To evaluate the effects of priming, seeds of Urochloa brizantha cv. Xaraés were primed under different osmotic potentials (ψ_s = 0.0; -0.5; -1.0 and -1.5 MPa) and temperatures (15 and 25°C) for 24, 48, 96 and 144 h. The germination percentage and germination speed index were evaluated, in a completely randomized design with four replications. It was verified that priming increases percentage and germination speed index of U. brizantha cv. Xaraés seeds cultivar, and these should be primed in water at 25°C (ψ_s = 0,0 MPa) for 85 h.

Key words: Brachiaria grass, germination, forage seeds.

INTRODUCTION

Cultivated pastures represent the basis of Brazilian beef cattle production (Laura et al., 2009; Paniago et al., 2014); the genus *Urochloa* (syn. *Brachiaria*), native from the African tropical savannas, is an important forage input for the country (Masetto et al., 2013; Batista et al., 2016a). According to IBGE (2007), there are approximately 95 million hectares cultivated with *Urochloa* species in Brazil, and occupied by *Urochloa brizantha* (Hochst. Ex A. Rich.) RD Webster (60 million ha), *U. decumbens* (Stapf) RD Webster (25 million ha), *U. humidicola* (Rendle) Morrone and Zuloaga (10 million ha). In this sense, Brazil is the largest seed producer, consumer and exporter of tropical forage species (Paniago et al., 2014; Batista et al., 2016b), exporting about 6,896.68 tonnes of seeds in 2017/2018 (MDIC, 2018).

For good quality pasture establishment, besides adequate management, it is very important to use seeds with high germinative power and vigor (Cardoso et al., 2014; Cardoso et al., 2015). Species with irregular seedlings emergence lead to delayed pasture establishment, which may favor weeds emergence in newly sown pastures (Cardoso et al., 2014). Among the forages, one of the main obstacles to uniform germination is seed dormancy (Batista et al., 2016b). This occurs in *U. brizantha* (Lacerda et al.,

*Corresponding author. E-mail: valdemir.laura@embrapa.br.

2010; Batista et al., 2016a), the most used forage species in Brazil and with the highest export seed volume (Gaspar-Oliveira et al., 2008).

Several studies evaluated the use of dormancy overcoming methods of *U. brizantha*, with emphasis on chemical scarification with sulphuric acid (Garcia and Cícero, 1992; Usberti and Martins, 2007; Gaspar-Oliveira et al., 2008; Cardoso et al., 2014). However, this method may reduce the seeds' physiological potential (Cardoso et al., 2014), as well as presenting risks to workers and the environment (Cardoso et al.. 2014), if it is not properly manipulated and disposed. A promising alternative that has not yet been studied to accelerate and standardize the species seed germination and, consequently, pasture stand uniformity, is seeds priming or osmotic conditioning (Batista et al., 2016a).

Priming treatments are directed to phases I and II of seeds imbibition, when the mechanisms of damaged macromolecules and cellular structures are repaired. During these hydration phases, required metabolic processes for seeds germination are activated without allowing the protrusion of the primary root, so, seeds do not reach phase III of imbibition (Contreiras-Rodrigues et al., 2009; Marcos-Filho, 2015). Therefore, priming can provide improvement in the expression of vigor, in addition to activating the physiological processes of germination, without the emission of the primary root (Contreiras-Rodrigues et al., 2009; Cardoso et al., 2015). The aim of this work was to evaluate the effects of priming on seeds germination and vigor of *U. brizantha* cv. Xaraés.

MATERIALS AND METHODS

This study was carried out at Embrapa Beef Cattle, Campo Grande (MS), Brazil, from February to October 2006. Priming was tested on *U. brizantha* cv. Xaraés seeds, produced in the 2004/2005 harvest. Priming tests were done to select the best treatment, using direct immersion method; the seeds were immersed in aqueous solutions with osmotic potentials (ψ_s) of: 0.0 (distilled water), -0.5; -1.0 and -1.5 MPa, obtained with PEG 6,000 solution (polyethylene glycol 6,000), according to Villela et al. (1991), under constant aeration. Five g of seeds of each cultivar was placed into 100 mL of PEG 6,000 solution, in the specific concentration assigned to each treatment. The priming was tested with time exposures of 24, 48, 96 and 144 h and under two temperature regimes: controlled temperature (15°C, in germination chamber) and at room temperature (± 25°C). After priming, seeds were washed in running water and put to dry at room ambient temperature for 24 h. As control treatment we used seeds without priming (untreated seeds). After drying, seeds were germinated in four repetitions of 100 seeds per treatment, on germitest paper and moistened with the equivalence of 2.5 times the substrate of distilled water. The seeds were incubated in germination chamber Biochemical Oxygen Demand (BOD) with photoperiod of 8 h and alternating temperature cycles of 20 (for 16 h) and 35°C (for 8 h) for 21 days, according to the Seed Analysis Rules (Brasil, 2009). The variables evaluated were:

Germination (%): Considered as germinated seeds the ones which presented at least 2.0 mm of seminal root (Juntila, 1976).

Germination speed index – GSI: determined according to the formula of Maguire (1962), $GSI = \Sigma(n/t)$

where: n= number of germinated seeds in the computed first, second, …, and last count; t= number of days from sowing to first, second, …, and last count.

For each variable, an analysis of variance and polynomial regression was performed, with the significance tested through an F test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Germination percentage of *U. brizantha* cv. Xaraés seeds was influenced by the three factors analyzed: temperature, exposure times and osmotic potential (Figure 1). At 15°C priming, significant relationships were found between germination percentage and the exposure times for -0.5 and 0.0 MPa osmotic potentials, being the best result found for conditioning in distilled water for 84 h. In this situation it was obtained the germination of 26.94%, about twice that obtained in the control treatment (untreated seeds) (Figure 1a). At 25°C priming, a significant relationship was obtained only in distilled water priming. At this temperature, highest germination (34.36%) was obtained in priming for 85 h, exceeding both the control treatment and priming in distilled water at 15°C (Figure 1b).

Priming in distilled water at 25°C resulted in an increase of 7.43% in germination compared to the temperature of 15°C. In this way, it is possible to infer that the temperature represented the preponderant factor to germination increase, since the time of exposure of the seeds to obtain the maximum germination was practically the same. Besides that, considering that the priming resulted in germination increase in relation to the control treatment, it is also possible to perceive that it acted as a treatment overcoming dormancy via humid heat. Contrary results were observed by Bonome et al. (2006), who observed that the reduction of the osmotic potential of the conditioning solution resulted in a germination percentage increase of *U. brizantha*. However, these results were lower than those of control treatment using scarified (83.5%) and unscarified (81.5%) seeds, evidencing that the seeds evaluated did not present primary dormancy.

Forage seeds are marketed based on their cultivation value, taking into account their germination percentage and purity (Brasil, 2008). However, germination results obtained in the laboratory do not always reproduce in the field. In this way, it is essential to identify vigor characteristics of the same, as the Germination Speed Index (GSI), since these are variable responses that will be closer to the real seeds performance in the field (Marcos-Filho, 2015).

GSI was influenced by the factors analyzed in a similar way to the percentage of germination (Figure 2). At 15°C

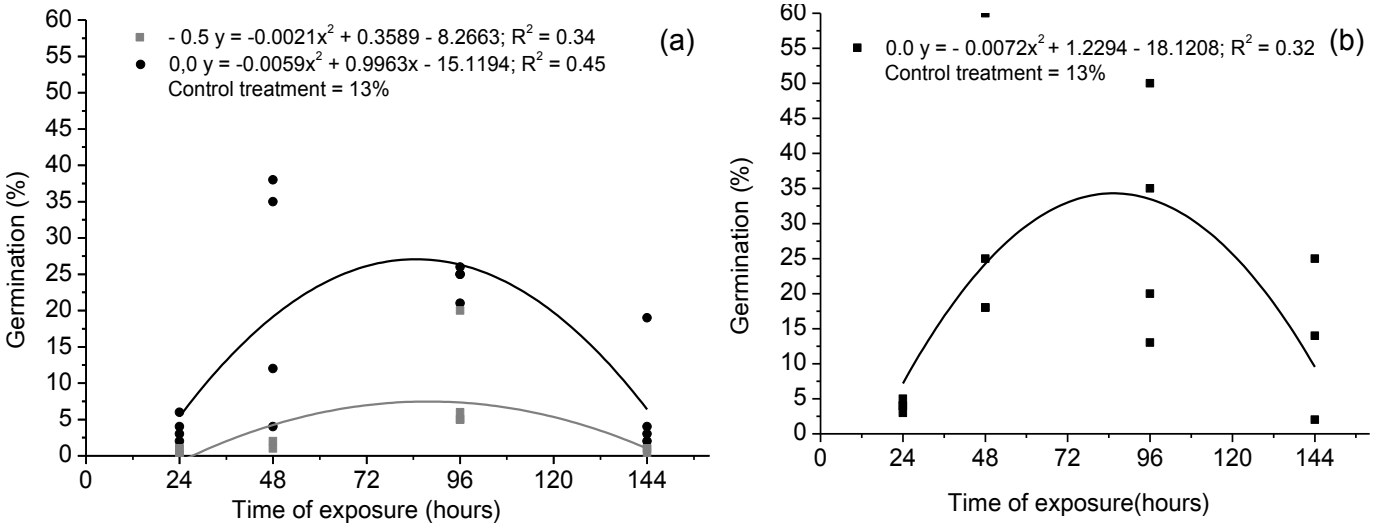

Figure 1. Germination percentage of *U. brizantha* primed seeds, Xaraés cultivar, under different osmotic potentials (ψ_s = 0.0, -0.5, -1.0 and -1.5 MPa), exposure times (24, 48, 96 and 144 h), conditioned at 15°C (a) squares represent 0.5 MPa and dots represent 0.0 MPa; and 25°C (b) squares represent 0.0 MPa. Non-significant relationships were not represented.

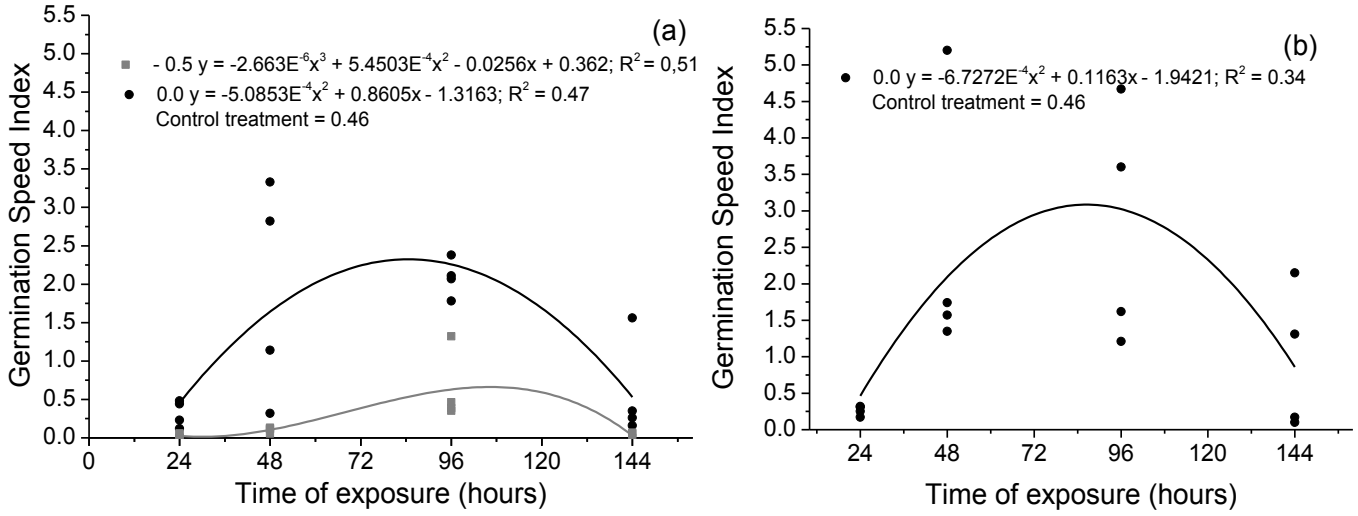

Figure 2. Germination Speed Index of *U. brizantha* primed seeds, Xaraés cultivar, under different osmotic potentials (ψ_s = 0.0, -0.5, -1.0 and -1.5 MPa), by different exposure times (24, 48, 96 and 144 hours), conditioned at 15°C (a) squares represent 0.5 MPa and dots represent 0.0 MPa; and 25°C (b) dots represent 0.0 MPa. Non-significant relationships were not represented.

priming, significant relationships were found between GSI and exposure times for -0.5 and 0.0 MPa potentials (Figure 2a). However, the highest GSI (2.32) was obtained for conditioning in distilled water for 85 h, a value that exceeded five times that obtained in the control treatment. At 25°C priming, a significant relationship was obtained only in the distilled water conditioning, with GSI

higher value of 3.1, surpassing both the control treatment (by 6.7 times) and the conditioning a in distilled water at 15°C (Figure 2b). Analyzing the physiological quality of *U. brizantha* cv. Xaraés seeds it is confirmed that priming at 25°C again worked as a treatment to overcome seeds dormancy. However, its performance on seed vigor was more expressive than on germination. Thus, the manifestation of priming on seed vigor would be decisive for plants establishment in field, resulting in a stand with faster and more uniform emergence.

Although most of the germination studies of *U. brizantha* recommend chemical scarification to overcome seed dormancy (Garcia and Cícero, 1992; Usberti and

Martins, 2007; Gaspar-Oliveira et al., 2008), the present study demonstrated the possibility of using moist heat treatment for this purpose. Our results demonstrated that the use of this treatment, simpler and environmentally safer, resulted in an increase in both germination and vigor of the evaluated seeds.

Conclusions

Priming increases percentage and germination speed index of *U. brizantha* cv. Xaraés seeds cultivar and these should be primed in water at 25°C (ψ_s = 0.0 MPa) for 85 h.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by FUNDECT/MS to perform this research and for maintaining scholarships of the first author and UNIPASTO and Fundação Manoel de Barros for the financial support.

REFERENCES

Batista TB, Cardoso E, Binotti FFS, Costa E, Sá ME (2016a). Priming and stress under high humidity and temperature on the physiological quality of *Brachiaria brizantha* cv. MG-5 seeds. Acta Scientiarum. Agronomy 38(1):123-127.

Batista TB, Binotti FFS, Cardoso ED, Costa E, Nascimento DM (2016b). Appropriate hydration period and chemical agent improve priming in brachiaria seeds. Pesquisa Agropecuária Tropical 46(3):350-356.

Bonome LTS, Guimarães RM, Oliveira JA, Andrade VC, Cabral OS (2006). Effect of osmoconditioning in seeds of *Brachiaria brizantha* cv. Marandu. Ciência e Agrotecnologia 30(3):422-428.

Brasil (2008). Instrução Normativa n. 30, de 21 de maio de 2008. Diário Oficial União, Brasília, DF, 23 mai. Seção 1: 45.

Brasil (2009). Regras para análise de sementes. Brasília: SNDA/ DNDV/CLAV, 2009. 395p.

Cardoso ED, Sá ME, Haga KI, Binotti FFS, Nogueira DC, Valério-Filho WV (2014). Physiological and performance in overcoming dormancy in seeds *Brachiaria brizantha* under artificial chemical treatment and aging. Semina: Ciências Agrárias 35(1):21-38.

Cardoso ED, Sá ME, Haga KI, Binotti FFS, Costa E (2015). Physiological quality and chemical composition of *Brachiaria brizantha* seeds as a function of priming. Revista de Agricultura Neotropical 2(2):42-48.

Contreiras-Rodrigues APD, Laura VA, Chermouth KS, Gadum J (2009). Osmopriming of parsley seeds (*Petroselinum sativum* Hoffm.) under different water potentials. Ciência e Agrotecnologia 33(5):1288-1294. Garcia J, Cícero SM (1992). Superação de dormência em sementes de *Brachiaria brizantha* cv. Marandu. Scientia Agricola 49(1): 91-13.

Gaspar-Oliveira CM, Martins CC, Nakagawa J, Cavariani C. Duração do teste de germinação de *Brachiaria brizantha* cv. Marandu (Hochst. ex A. Rich.) Stapf (2008). Revista Brasileira de Sementes 30(3):30-38.

IBGE (2007). Resultados preliminares do Censo Agropecuário confirmam expansão da fronteira agrícola na região Norte, 2007. Available in: <http://www.ibge.gov.br/home/presidencia/noticias/noticia_visualiza.php?id_noticia=1064&id_pagina=1> Accessed in: 15 jul. 2010.

Juntila O (1976). Seed and embryo germination in *S. vulgaris* and *S. reflexa* as effects by temperature during seed development. Physiologia Plantarum 29(2):264-268.

Lacerda MJR, Cabral JSR, Sales JF, Freitas KR, Fontes AJ (2010). Seed dormancy-breaking of *Brachiaria brizantha* cv. Marandu. Semina: Ciências Agrárias 31(4):823-828.

Laura VA, Contreiras-Rodrigues APD, Arias ERA, Chermouth KS, Rossi T (2009). Physical and physiological quality of seeds of Brachiaria grass commercialized in Campo Grande (Brazil). Ciência e Agrotecnologia 33(1):326-332.

Maguire JD (1962). Speeds of germination-aid selection and evaluation for seedling emergence and vigor. Crop Science 2(2):176-177.

Marcos-Filho J (2015). Fisiologia de Sementes de Plantas Cultivadas. 2ed; - Londrina, PR: Abrates 660 p.

Masetto TE, Ribeiro DM, Rezende RKS (2013). Germinação de sementes de *Urochloa ruziziensis* em função da disponibilidade hídrica do substrato e teor de água das sementes. Pesquisa Agropecuária Tropical 43(4):385-391.

Paniago BC, Pereira SR, Contreiras-Rodrigues APD, Laura VA (2014). Dormência pós-colheita de sementes de *Urochloa humidicola* (Rendle) Morrone & Zuloaga. Informativo Abrates 24(1):22-26.

Usberti R, Martins L (2007). Sulphuric acid scarification effects on *Brachiaria brizantha*, *B. humidicola* and *Panicum maximum* seed dormancy release. Revista Brasileira de Sementes 29(2):143-147.

Villela FA, Doni-Filho L, Sequeira EL (1991). Table of osmotic potential as a function of polyethilene glycol 6000 concentration and temperature. Pesquisa Agropecuária Brasileira 26(11-12):1957-1968.

Related Journals: