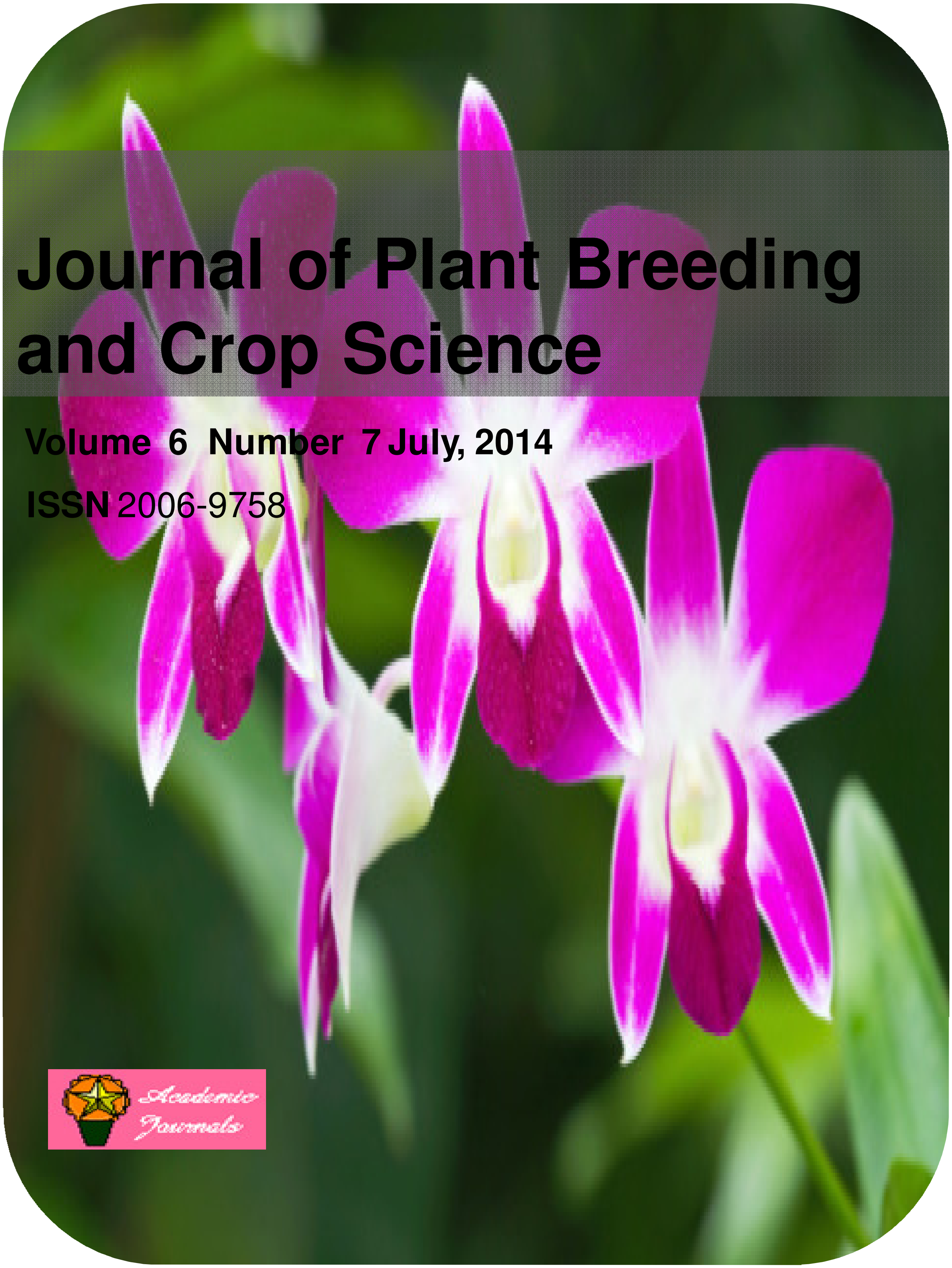

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Full Length Research Paper

Evaluation of highland maize (*Zea mays* L.) cultivars for polyethylene glycol (PEG) induced moisture stress tolerance at germination and seedling growth stages

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A laboratory experiment was performed at Ambo University, Department of Plant Sciences in March 2014, to evaluate the effect of moisture stress on germination and seedling growths of highland maize cultivars. The experiment was arranged factorially in completely randomized design with three replications. Five highland maize cultivars (Hora, Wenchi, Jibat, Argene and Wabi) were exposed to six levels of water stress (0, 60, 120, 180, 240 and 300 g/L Polyethylene glycol 6000). The result revealed that no significant interactions exist between maize cultivars and moisture stress. However, cultivars varied significantly for germination percentage and rate, shoot and root lengths, root number, and shoot and root fresh weight. Increase in PEG 6000 concentrations decreased germination percentage and rate, where as shoot and root lengths decreased beyond 60 g/l. No significant differences were observed among 60, 120 and 180 g/L for shoot and root fresh and dry weights, and seedling fresh and dry weights. Maximum root number, root-to-shoot ratio and tolerance index was observed at 120 and 180, 240 and 60 g/L PEG, respectively. Hence, highland maize cultivars showed differential response in terms of germination and seedling growth with increased moisture stress, and increase in PEG 6000 reduced germination and seedling growth beyond 60 g/L.

Key words: Germination, maize, seedling growth, stress, tolerance.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop in terms of acreage, production, yield, distribution and adaptation in Ethiopia. Estimates of annual maize productivity per hectare reach 22 quintals, and the area coverage is 1.77 million hectares (CSA, 2011) occupying first place in total grain production and yield per hectare; although the national average yield is below the world average. The causes for low productivity of maize are abiotic and biotic constraints. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bayoumi et al., 2008). The causes for low productivity of maize are abiotic and biotic constraints. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most

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major crop plants by more than 50% (Bayoumi et al., 2008). The causes for low productivity of maize are abiotic and biotic constraints. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bayoumi et al., 2008) which are widespread problems around the world (Soltani et al., 2006). Water stress results in reduced plant growth and yield by affecting almost every developmental stage of the plant. However, damaging effects of moisture stress was more pronounced when it coincided with various phenophases such as germination; seedling stage and flowering (Khayatnezhad et al., 2010).

Field experiments related to water stress have been difficult to handle due to significant environmental or drought interactions with other abiotic stresses (Rauf, 2008). Establishing conditions of drought stress using different osmotic materials to create the osmotic potential is considered as one of the best methods to study the effects of moisture stress on germination. Hence, an alternative approach is to induce water stress through polyethylene glycol (PEG) solutions for screening the germplasm (Khodarahmpour, 2011; Rajendran et al., 2011). Water sensitive stages may be exploited to discriminate genotypes on the basis of their resistance to water stress. Among these critical stages, water stress induced during seedling stage has been exploited in various crop species to screen germplasm or breeders populations (Bibi et al., 2012; Khayatnezhad, 2010). The current study was therefore made to evaluate the effect of moisture stress on germination and seedling growth of highland maize cultivars.

MATERIALS AND METHODS

Laboratory experiment was conducted in the Department of Plant Sciences, Ambo University, Ethiopia, to evaluate moisture stress effect on germination and seedling growth of maize cultivars. The experiment was arranged factorially in a completely randomized design with three replications. Five highland maize cultivars (Hora,Wenchi, Jibat, Argene and Wabi) were exposed to six levels of water stress (0, 60, 120, 180, 240 and 300 g/L PEG 6000) in the experiment, de-ionized water was used for the control treatment. Polyethylene glycol solution (PEG 6000) was dissolved with the respective treatment amount at 25°C with deionized water. Seeds treated with fungicides were used in the experiment. Ten seeds were uniformly placed on Watman filter paper in the Petri dish (9.5 cm diameter) using a forceps for each treatment, and well soaked by adding 22 ml of the respective solutions. All the Petri dishes were covered with lids and kept at room temperature (22 ± 2°C). Germination continued for 10 days, and germinated seeds were counted daily. The emergence of 2 mm radical was the criteria of germination. After 10 days, parameters such as percent germination and rate of germination were calculated according to ISTA (1999); and root and shoot lengths of seedling were measured using a scale. Root and shoot dry weights were recorded after oven drying for 24 h at 80°C. The seedling tolerance index (STI) was determined based on the methods described by Iqbal and Rahmati (1992).

Statistical analysis of the data was performed employing One-Way ANOVA using SAS statistical software (Version 9). Based on the ANOVA results, mean separations were performed by Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

Germination, and shoot and root lengths

The analysis of variance indicated no significant interaction between maize cultivars and moisture stresses. However, cultivars varied significantly for germination percentage and rate. Jibate cultivar gave maximum germination percentage (92.5%), while Hora resulted in high germination rate (1.91 plants/day). Nevertheless, no significant difference was observed between Hora, Wenchi and Jibat in germination percentage and rate (Table 1). The lowest germination percentage (76.9%) and rate (1.55 plants/day) was recorded in Wabi cultivar. This finding is in agreement with the trends of Almaghrabi (2012) findings, who observed significant differences in response to drought stress on germination of wheat cultivars.

Moisture stress significantly influenced not only germination percentage and rate, but also shoot and root lengths. Germination percentage and rate were highest in control treatment and tended to decrease as the moisture stress increased using PEG (Table 2). Reduction in germination with moisture stress is attributed to lower infusibility of water through the seed coat and initial water imbibition of the seed under stress condition (Bahrami et al., 2012) and decreased external water potential. Decrease in seed germination under water stress condition could also be due to metabolic disorders such as slow hydrolysis of substrate compounds in endosperm or cotyledons and/or slower transportation of hydrolyzed material to developing embryo axis (Ayaz et al., 2000). Maximum shoot and root lengths were recorded at 60 g/L PEG stress level, but further increase in PEG concentration decreased shoot and root lengths significantly. Ghajari and Zeinali (2003) also observed an increase in shoot and radicle lengths until -0.2 MPa when using PEG-6000. Similar results also have been reported by Boureima et al. (2011) who stated that root length increased by 19.94% at -0.5 MPa in comparison with controls. Moderate drought stress increased root lengths of pearl millet cultivars by 15.8% (Radhouane, 2008). The development of the root system in response to water deficit suggests that the expression of certain genes controlling root formation is stimulated by drought conditions (Badiow et al., 2004). However, the reduction of radicle length due to excess exposure for moisture stress could be due to a cessation in cellular division and elongation at root level.

Shoot and root fresh and dry weights

The results of the study revealed that maize cultivars differed significantly in shoot and root fresh weights.

Table 1. Germination, and shoot and root lengths of maize cultivars as influenced by moisture stress.

Cultivar	Germination (%)	Germination rate (Plant/day)	Shoot length (cm)	Root length (cm)
Hora	87.2^{ab}	1.91^{a}	$4.10\ (2.795)^{a}$	$10.69\ (4.14)^{ab}$
Wenchi	87.8^{ab}	1.65^{ab}	$3.84\ (2.788)^{ab}$	$10.84\ (4.18)^{ab}$
Jibat	92.5^{a}	1.78^{ab}	$3.70\ (2.785)^{ab}$	$9.92\ (4.04)^{b}$
Wabi	76.9c	1.55^{b}	$3.45\ (2.715)^{ab}$	$11.28\ (4.23)^{a}$
Argene	83.3^{b}c	1.59^{b}	$3.29\ (2.632)^{b}$	$10.11\ (4.06)^{ab}$
SEm(±)	2.03	0.087	0.084	0.094
CV (%)	11.9	24	11.6	6.1

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed data.

Table 2. Effect of moisture stress on germination, and shoot and root length of maize.

Moisture stress [PEG 6000 conc. (g/L)]	Germination (%)	Germination rate (Plant/day)	Shoot length (cm)	Root length (cm)
0	99.3^{a}	2.79^{a}	$5.61\ (3.35)^{ab}$	$14.14\ (\ 4.75)^{b}$
60	96.7^{ab}	2.24^{b}	$6.28\ (3.50)^{a}$	$15.27(\ 4.90)^{a}$
120	$96\ ^{ab}$	1.95^{b}	$5.06\ (3.23)^{b}$	$13.73\ (4.70)^{b}$
180	90.7^{b}	1.52^{c}	$3.64\ (2.90)^{c}$	$12.33(4.51)\ ^{c}$
240	79.3^{c}	1.03^{d}	$0.98\ (1.94)^{d}$	$4.99\ (3.22)^{d}$
300	53.3^{d}	0.67^{e}	$0.49\ (1.54)^{d}$	$2.97(\ 2.70)^{d}$
SEm(±)	2.03	0.087	0.084	0.094
CV (%)	11.9	24	11.6	6.1

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed data.

Table 3. Effect of moisture stress on shoot and root fresh and dry weights of maize cultivars.

Cultivars	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
Hora	$0.116\ (1.30)^{b}$	$0.159\ (1.37)^{ab}$	$0.0279\ (\ 1.161)^{a}$	$0.051\ (1.217)^{a}$	$0.269\ \ (1.477)\ ^{a}$	$0.0789\ (1.263)^{a}$
Wenchi	$0.168\ (1.37)^{a}$	$0.164\ (1.38)^{ab}$	$0.0321\ (1.167)^{a}$	$0.059\ (1.22)^{a}$	$0.332\ \ (1.540)\ ^{a}$	$0.0914\ (1.281)^{a}$
Jibat	$0.122\ (1.32)^{ab}$	$0.151\ (1.36)^{b}$	$0.0263\ (1.152)^{a}$	$0.0419\ (1.183)^{a}$	$0.273\ \ (1.486)\ ^{a}$	$0.682\ (1.242)^{a}$
Wabi	$0.139\ (1.35)^{ab}$	$0.150\ (1.36)^{b}$	$0.0270\ (1.159)^{a}$	$0.0467\ (1.189)^{a}$	$0.289\ \ (1.505)\ ^{a}$	$0.0737\ (1.252)^{a}$
Argene	$0.134\ (1.33)^{ab}$	$0.197\ (142)^{a}$	$0.0269\ (1.158)^{a}$	$0.\ 0633\ (1.225)\ ^{a}$	$0.331\ \ (1.539)\ ^{a}$	$0.0902\ (1.273)^{a}$
SEm(±)	0.016	0.015	0.007	0.011	0.021	0.012
CV (%)	6.3	5.2	3.0	6.0	6	4.9

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed data.

Similarly, Bibi et al. (2012) reported that drought has drastically affected fresh shoot and root weight in some cultivars of sorghum, wheat, maize and sunflower. However, shoot and root dry weights, and seedling fresh and dry weights of maize cultivars were not significantly different (Table 3). Cultivar Wenchi showed maximum shoot, root and seedling fresh and dry weights. Results of the current study in relation to the existence of variability among cultivars were in agreement with other experiments in chickpea (Kalefetoglu et al., 2009) and in wheat (Almansouri et al., 2001; Soltani et al., 2006).

Moisture stress levels induced by PEG 6000 concentrations significantly decreased shoot, root and seedling fresh and dry weights as PEG levels increased beyond 120 g/L (Table 4). Positive increment was observed on fresh and dry weights up to 120 g/L PEG levels. However, no significant differences were observed between 60 and 120 g/L treatments. An increase in fresh

Table 4. Effect of moisture stress induced by PEG on shoot and root fresh and dry weights of maize.

PEG 6000 Conc. (g/L)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
0	0.162 (1.378)^{b}	0.111(1.32)^{b}	0.0367(1.190)^{b}	0.0454 (1.21)^{b}	0.273 (1.50)^{b}	0.082(1.28)^{b}
60	0.221 (1.462)^{a}	0.23 (1.47)^{a}	0.0468 (1.215)^{a}	0.0718 (1.26)^{a}	0.45 (1.66)^{a}	0.119 (1.34)^{a}
120	0.2 (1.447)^{a}	0.279 (1.53)^{a}	0.0369 (1.192)^{ab}	0.0922 (1.30)^{a}	0.48 (1.69)^{a}	0.129 (1.36)^{a}
180	0.173 (1.413)^{ab}	0.249 (1.49)^{a}	0.0312 (1.175)^{ab}	0.0788 (1.25)^{a}	0.422 (1.64)^{a}	0.11(1.32)^{a}
240	0.04 (1.189)^{c}	0.077 (1.27)^{b}	0.0135 (1.106)^{c}	0.0192 (1.13)^{c}	0.116 (1.34)^{c}	0.033 (1.18)^{c}
300	0.023(1.123)^{d}	0.031(1.17)^{c}	0.0068 (1.066)^{c}	0.0075 (1.08)^{c}	0.054 (1.22)^{c}	0.014 (1.11)^{c}
SEm(±)	0.016	0.015	0.007	0.011	0.021	0.012
CV (%)	6.3	5.2	3.0	6.0	6.0	4.9

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed data.

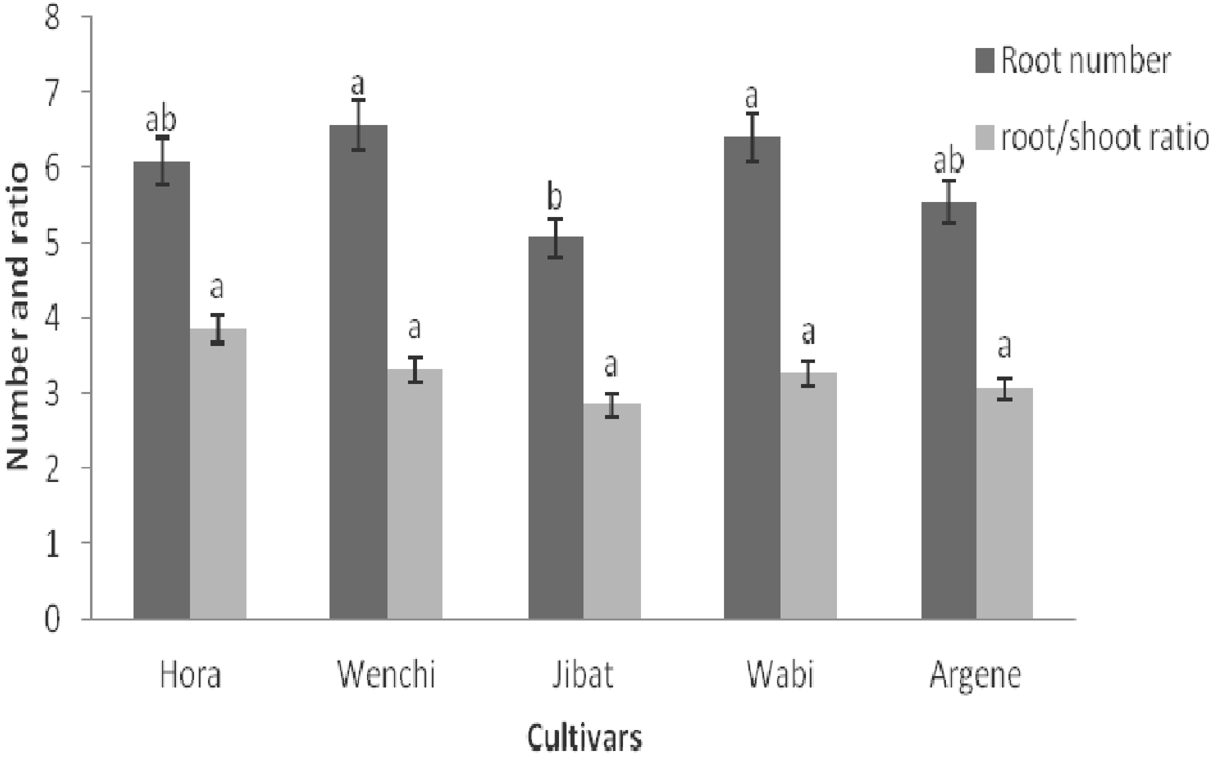

Figure 1. Moisture stress effect on root number and root-to-shoot ratio of maize cultivars.

and dry weights of shoot under stress could be attributed to the accumulation of organic and inorganic solutes and due to the higher growth because of osmotic adjustment. Almaghrabi (2012) reported that PEG caused a greater reduction in fresh and dry weights of shoot and root at higher concentrations compared with control.

Root number, root-to-shoot ratio, and tolerance index

Maize cultivars differed significantly in root number, but not in root-to- shoot ratio. Maximum root number was observed on cultivars Wenchi and Wabi, while the lowest was recorded on Jibat (Figure 1). Wenchi and Hora cultivars exhibited maximum and minimum tolerance indices, respectively to moisture stresses (Figure 2). High number of roots increases the ability of the crop to extract moisture from the growing rhizosphere under moisture stress condition.

Significant difference was observed in root number with increase in the levels of moisture stress (Figure 3). Treatments 120 and 180 g/L PEG gave the maximum root number, while the least was observed with 300 g/L PEG level. Treatment 240 g/L PEG recorded maximum

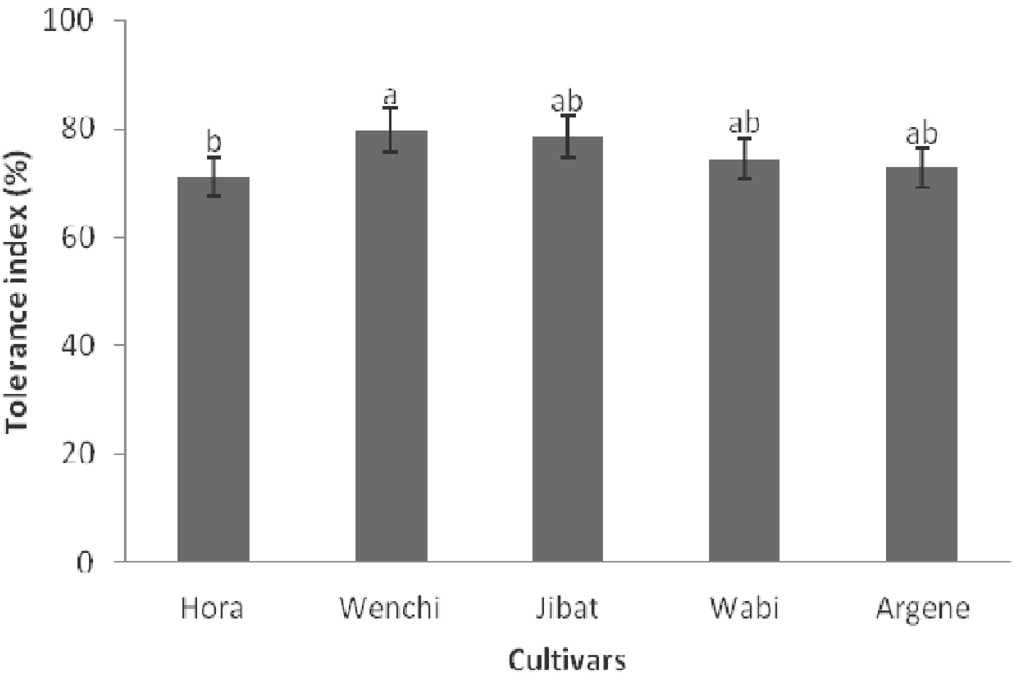

Figure 2. Effect of moisure stress on tolerance index of maize cultivars.

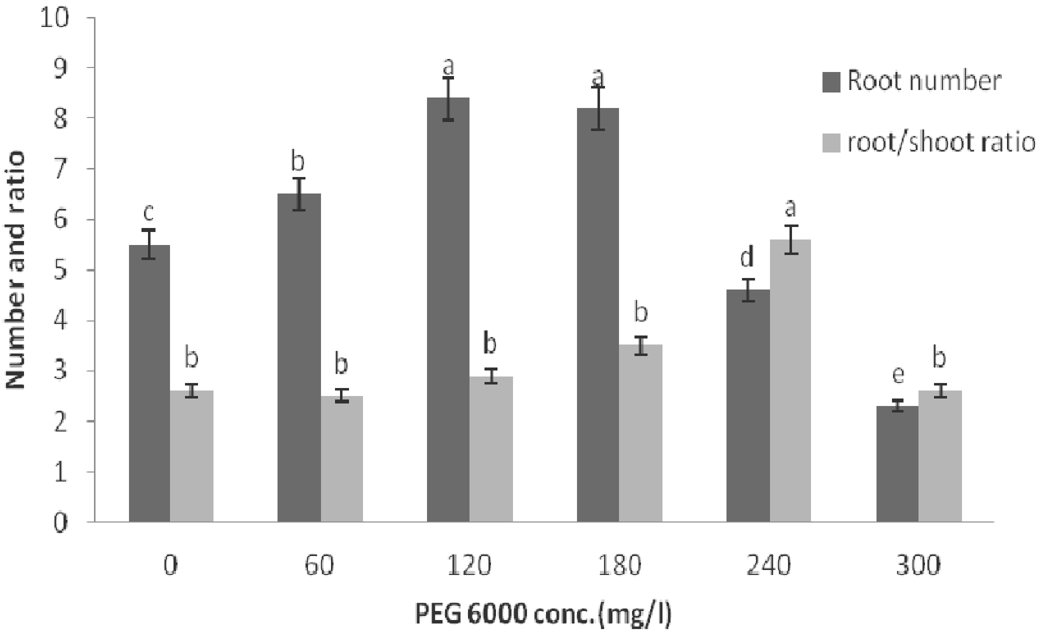

Figure 3. Moisture stress effect on root number and root-to-shoot ratio of maize.

root-to-shoot ratio, which could be due to the minimal shoot growth compared to the root growths. Tolerance index decreased with increase in PEG concentrations beyond 60 g/L (Figure 4). Maximum tolerance index was observed in 60 g/L and the least was observed in 300 g/L treatments. Similar results were reported in wheat by Almaghrabi (2012).

Conclusion

Highland maize cultivars showed differential response to

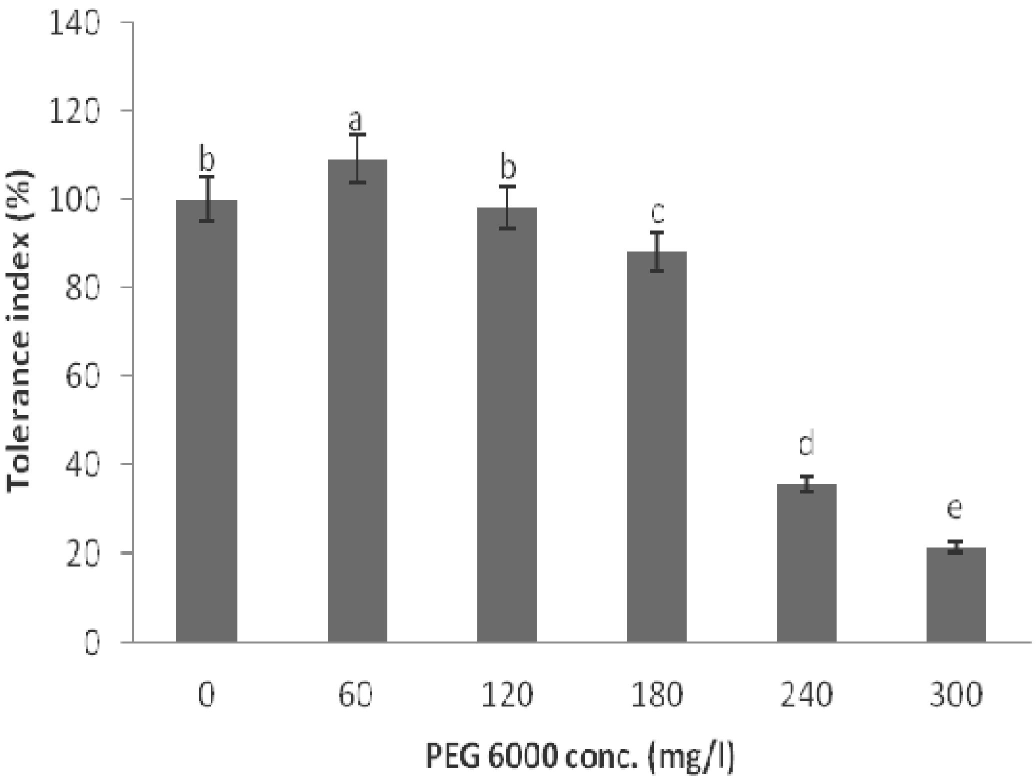

Figure 4. Effect of moisure stress on tolerance index of maize.

water stress at germination and early seedling growth stages. Cultivars varied for germination percentage and rate, shoot and root lengths, root number, and shoot and root fresh weight. Cultivar Wenchi was found relatively tolerant to moisture stress induced by PEG. Increase in PEG 6000 concentrations decreased germination percentage and rate, while shoot and root lengths, and shoot fresh and dry weights decreased beyond 60 g/L. Root fresh and dry weights increased up to 120 g/L PEG, but further increase in stress negatively influenced cultivars tolerance.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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Full Length Research Paper

Combining ability studies of pigeonpea cytoplasmic male sterile (CMS) lines with an obcordate leaf marker

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Hybrid pigeonpea [*Cajanus cajan* (L.) Millsp.] breeding technology based on cytoplasmic-genetic male sterility (CMS) was recently developed ICRISAT, Patancheru in India. A program was initiated to track the purity of female parental lines by incorporating an obcordate leaf shape marker in established male sterile A-lines. Seven obcordate A-lines developed by backcrossing and selection were crossed with four known fertility restorers in line x tester mating design to study their general and specific combining ability. Higher magnitude of specific combining ability (SCA) effect showed that, hybrid yield was under the control of non-additive genes. Among A-lines, ICPA 2204 was the best general combiner. Among testers, ICPL 20116 was the best general combiner. Among hybrids, ICPA 2208 x ICPL 20108 a cross between high general combining ability (GCA) parents was the best with positive significant SCA effect and higher mean performance for grain yield, 100-seed mass, number of seeds/pod and resistance to fusarium wilt disease. The success of this technology will help to address the issue of seed purity to some extent.

Key words: Combining ability, line × tester, general combining ability (GCA) and specific combining ability (SCA), obcordate leaf shape, pigeonpea.

INTRODUCTION

In order to achieve a breakthrough in the productivity of pigeonpea, hybrid breeding technology based on cytoplasmic genetic male sterility was developed at ICRISAT (Saxena, 2008). Significant yield gains with improved disease and drought resistance in the hybrids over traditional cultivars are likely to help in enhancing production and productivity of pigeonpea. To achieve this mission, it is important to establish a stable and robust hybrid seed production technology that will fulfil the ever increasing demand for quality hybrid seed. The genetically uniform parental lines and commercial hybrids are necessary in production and marketing of quality hybrid crops. Quality control of hybrid seeds is traditionally done by Grow out Test (GoT) in most of the crops. In pigeonpea this process is more resource intensive in terms of time and labour due to its long generation time. Considering this constraint, efforts were made to incorporate an easily identifiable morphological marker [naked eye polymorphism (NEP)] in female parents to track the purity of the female parent and hybrids. Although morphological markers are limited in nature but their assays neither require sophisticated

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Table 1. Combining ability analysis for different traits.

Source	df	Mean sum of squares Days to 50% flowering	Days to 75% maturity	Plant height (cm)	Number of seeds/pod	100-seed mass (g)	Grain yield (kg/ha)
Replication	1	5.79	28.57	429.02	0.00	0.12	4350.53
Crosses	27	26.38*	30.02*	76.90	0.04	0.88	198365.24*
Lines	6	14.35	41.37	88.24	0.06	1.78	166296.15
Testers	3	114.40**	71.43**	220.69	0.01	2.15	292601.67*
Line x Tester	18	15.72	19.35	49.16	0.04	0.38	193348.05*
Error	27	11.16	12.83	61.43	0.03	0.74	89625.19
Variance of GCA		4.42	3.37	9.57	0.00	0.14	3281.71
Variance of SCA		2.28	3.26	-6.13	0.01	-0.18	51861.43
GCA/SCA		1.94	1.03	-1.56	0.00	-0.80	0.06

*, ** Significant at 5 and 1% level of probability, respectively.

equipment nor complicated procedures (Singh and Singh, 1992). The obcordate leaf shape used as NEP is a highly heritable trait with single recessive genetic control. To develop hybrid technology based on NEP, the obcordate leaf marker was transferred to A-lines through backcrossing (Saxena et al., 2011). The objective of this study was to identify obcordate male sterile lines with good general and specific combining ability for use in hybrid breeding program.

MATERIALS AND METHODS

Seven cytoplasmic male sterile (CMS) lines with obcordate leaf marker and their corresponding B-lines were developed by backcrossing two established male sterile lines ICPA 2047 and ICPA 2048 with obcordate leaf donor ICP 5529. These A-lines designated as ICPA 2200, ICPA 2201, ICPA 2202, ICPA 2203, ICPA 2204, ICPA 2206 and ICPA 2208 were crossed with four known male fertility restorers (ICPL 20116, ICPL 87119, ICPL 20108 and ICPL 20093) in a line x tester mating design. A field experiment was conducted with 28 F_1 hybrids and three standard checks ICPL 87119 (Asha), ICPH 2671, and ICPH 2740 in a randomized complete block design with two replications. The experiment was conducted at Patancheru in 2012 rainy season. Each plot consisted of two rows of 4 m length with inter and intra row spacing of 75 and 30 cm, respectively. To avoid border effect, one border row was planted at each side of the plot and first and last plant from each row was excluded from the plot yield and yield kg/ha was calculated considering net plot area 5.4 m^2. All the hybrids and their parents were also grown in single row plots in disease sick nursery for assessing their reaction to fusarium wilt and sterility mosaic diseases. Fusarium wilt incidence was recorded based on the number of plants wilted out of total plants available in a plot, and expressed in percentage. The incidence of sterility mosaic was studied using leaf stapling method. In this technique 10 to 15 day old seedlings were stapled with leaves infected with eriophyid mite (*Aceria cajani*) that carries sterility mosaic virus. Sterility mosaic incidence was recorded based on the number of plants infected out of total plants available in a plot, and expressed in percentage. The agronomic practices included basal application at 100 kg/ha of di-ammonium phosphate. Pre-emergence herbicide application using pendimenthalin and paraquat dichloride at 2 and 4 l/ha was done to control weeds. Two hand weedings, two irrigations at flower initiation and pod development, and three sprays of pesticides (acephate and spinosoid at 1 kg/ha and 0.2 L/ha, respectively) were done to control pod borer complex. Data were recorded on days to 50% flowering, days to 75% maturity, plant height, plant stand at the time of harvesting, number of seeds/pod, 100-seed mass and seed yield (kg/ha). The statistical analysis was performed using AGROBASE GEN-II software.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) along with estimates of general combining ability (GCA) and specific combining ability (SCA) variances for six characters is presented in Table 1. The analysis revealed significant differences among testers and crosses for days to 50% flowering, days to 75% maturity, and grain yield. Variance due to lines x testers was also significant for grain yield indicated the importance of specific combining ability. These results are in agreement with those of Kumar et al. (2003) and Phad et al. (2007). The mean squares due to testers were of larger magnitude than those of lines and line x tester for all the characters except number of seeds/pod indicating greater diversity among the testers than the lines and this is expected because the seven lines were derived using two females and one donor parent.

General and specific combining ability

The magnitude of SCA variance for grain yield was much greater than that of GCA, suggesting the preponderance of non-additive gene action. This observation is in agreement with the results of Baskaran and Muthiah (2007), Waghela et al. (2009) and Acharya et al. (2009). The rest of the characters except number of seeds per pod showed greater magnitude of GCA than SCA and it indicated that these traits were under the control of additive gene action. Similar results were also reported by Khorgade et al. (2000) and Thiruvengadam and Muthiah

Table 2. Estimates of general combining ability effects for different characters.

Parents	Days to 50% flowering	Days to 75% maturity	Plant height (cm)	Number of seeds/pod	100-seed mass (g)	Grain yield (kg/ha)
Lines						
ICPA 2200	-1.018	-3.30**	-2.68	0.06	-0.18	77.51
ICPA 2201	-0.768	-2.68*	1.07	-0.14*	-0.41	-266.04*
ICPA 2202	-1.518	-0.80	6.70*	0.11	-0.26	-62.72
ICPA 2203	-0.518	1.70	-1.43	-0.07	-0.27	21.31
ICPA 2204	1.607	1.70	-3.30	-0.01	0.33	188.71
ICPA 2206	1.982	2.32	-0.18	-0.02	0.93**	-38.92
ICPA 2208	0.232	1.07	-0.18	0.06	-0.16	80.14
Testers						
ICPL 20116	-3.11**	-2.14*	-2.77	0.03	0.56	154.73
ICPL 87119	-0.32	0.71	0.45	0.00	-0.22	-59.14
ICPL 20108	-0.39	-1.43	5.45*	0.01	-0.08	76.08
ICPL 20093	3.82**	2.86**	-3.13	-0.04	-0.26	-171.68*
SE (Lines)	1.093	1.172	2.565	0.054	0.282	97.994
SE (Testers)	0.773	0.829	1.814	0.038	0.199	69.292

*, ** Significant at 5 and 1 % level of probability, respectively.

(2012) for days to 50% flowering and number of seeds/pod.

The estimates of GCA effects (Table 2) revealed that male sterile lines ICPA 2204, ICPA 2208, ICPA 2200 and ICPA 2203 had positive GCA effects for grain yield. For days to 50% flowering, ICPA 2200 and ICPA 2201 showed highly significant negative GCA effects. Among the testers, ICPL 20116, ICPL 20108 and ICPL 20093 were superior parents considering their GCA effects for yield and early maturity. ICPA 2206 exhibited highly significant positive GCA effect for the character 100-seed mass. Lines ICPA 2204, ICPA 2208, ICPA 2203 and testers ICPL 20116 and ICPL 20108 were selected based on their higher GCA effects for yield and yield contributing characters.

SCA effect is generally considered the best criteria for selection of superior hybrid combination. In the present investigation no cross combination was found good for all the characters studied (Table 3). However, hybrids ICPA 2204 x ICPL 20093, ICPA 2208 x ICPL 20108 and ICPA 2203 x ICPL 20116 showed significant positive SCA effects for grain yield. For earliness, hybrid ICPA 2202 x ICPL 20093 showed highly significant negative SCA effect, whereas ICPA 2206 x ICPL 87119 and ICPA 2200 x ICPL 20108 showed significant negative SCA effects suggesting their importance in developing short duration hybrids. The SCA effects for plant height and 100-seed mass were non-significant indicating little or no variation among the parental lines for these traits. This was further confirmed with the results obtained in analysis of variance (Table 1). Hybrids ICPA 2201 x ICPL 20116 and ICPA 2203 x ICPL 20093 exhibited significant positive SCA effects for number of seeds/pod. Considering SCA effects for yield and yield contributing traits, hybrids ICPA 2204 x ICPL 20093, ICPA 2208 x ICPL 20108, ICPA 2203 x ICPL 20116 were found promising for improving grain yield.

Vanniarajan et al. (1999) reported that some of the cross combinations having parents with high x low and low x high GCA effects for grain yield also produced significant SCA effects. It was observed for the crosses, ICPA 2204 x ICPL 20093 and ICPA 2203 x ICPL 20116 for grain yield kg/ha. This high SCA effect of high x low combinations indicated the operation of additive x non-additive gene effects and hence these crosses can be utilized in heterosis breeding. The cross ICPA 2208 x ICPL 20108 showed significant SCA effects when both the parents also had average general combining ability. It revealed the operation of non-additive gene effects. Similar results were also reported by Devi et al. (2011) for grain yield and its component characters.

Heterosis and per se performance

Standard heterosis was estimated over control ICPL 87119 (Asha) a high-yielding pigeonpea variety, resistant to fusarium wilt and sterility mosaic diseases, identified for release in the central and south zones of India in 1992. The variety has medium maturity duration (180 days) with average seed size of 10.7 g and has been recommended for release in sterility mosaic endemic areas in deep block soils. Hybrid ICPA 2200 x ICPL 20108 expressed significant negative heterosis for maturity (Table 4) which is a desirable characteristic. Plant height recorded significant increase in only one

Table 3. Estimates of specific combining ability effects in 28 hybrids for different characters.

Hybrids	Days to 50% flowering	Days to 75% maturity	Plant height (cm)	Number of seeds/pod	100 seed mass (g)	Grain yield (kg/ha)
ICPA 2200 X ICPL 20116	-0.27	-0.98	-1.61	-0.03	-0.16	96.19
ICPA 2200 X ICPL 87119	3.95	3.66	-2.32	-0.05	-0.32	106.31
ICPA 2200 X ICPL 20108	-3.98*	-4.20*	5.18	-0.06	-0.06	-140.36
ICPA 2200 X ICPL 20093	0.30	1.52	-1.25	0.14	0.54	-62.15
ICPA 2201 X ICPL 20116	-0.52	-4.11	2.14	0.22*	0.06	-158.31
ICPA 2201 X ICPL 87119	0.70	0.54	-8.57	0.05	-0.25	67.01
ICPA 2201 X ICPL 20108	1.77	2.68	1.43	0.04	0.12	53.69
ICPA 2201 X ICPL 20093	-1.95	0.89	5.00	-0.31	0.06	37.60
ICPA 2202 X ICPL 20116	2.73	4.02	6.52	-0.08	-0.34	-182.57
ICPA 2202 X ICPL 87119	2.45	1.16	0.80	0.00	0.46	57.00
ICPA 2202 X ICPL 20108	0.02	0.80	-6.70	-0.06	-0.03	252.23
ICPA 2202 X ICPL 20093	-5.20*	-5.98**	-0.63	0.14	-0.09	-126.66
ICPA 2203 X ICPL 20116	-0.27	-0.98	-2.86	0.01	0.33	381.94*
ICPA 2203 X ICPL 87119	-1.05	1.16	3.93	-0.11	0.07	-425.14*
ICPA 2203 X ICPL 20108	-0.48	-1.70	3.93	-0.12	0.08	174.84
ICPA 2203 X ICPL 20093	1.80	1.52	-5.00	0.22*	-0.48	-131.65
ICPA 2204 X ICPL 20116	0.61	1.52	-3.48	-0.10	0.23	66.94
ICPA 2204 X ICPL 87119	-1.68	-1.34	3.30	0.08	-0.18	209.36
ICPA 2204 X ICPL 20108	-1.61	0.80	-1.70	-0.03	-0.37	-758.21**
ICPA 2204 X ICPL 20093	2.68	-0.98	1.88	0.06	0.33	481.90**
ICPA 2206 X ICPL 20116	-2.27	-1.61	3.39	0.01	0.73	78.38
ICPA 2206 X ICPL 87119	-3.55	-4.46*	-2.32	0.04	0.02	56.05
ICPA 2206 X ICPL 20108	2.02	2.68	-4.82	0.08	-0.37	-30.62
ICPA 2206 X ICPL 20093	3.80	3.39	3.75	-0.13	-0.38	-103.81
ICPA 2208 X ICPL 20116	-0.02	2.14	-4.11	-0.03	-0.84	-282.58
ICPA 2208 X ICPL 87119	-0.80	-0.71	5.18	0.00	0.21	-70.61
ICPA 2208 X ICPL 20108	2.27	-1.07	2.68	0.14	0.62	448.42*
ICPA 2208 X ICPL 20093	-1.45	-0.36	-3.75	-0.11	0.01	-95.23

*, ** Significant at 5 and 1 % level of probability, respectively.

cross ICPA 2202 x ICPL 20116. Number of seeds/pod showed significant heterosis (11.1%) in crosses ICPA 2202 x ICPL 20093 and ICPA 2208 x ICPL 20108 over the standard check. Considering per se performance, high positive heterosis was revealed for crosses ICPA 2208 x ICPL 20108 (60.4%), ICPA 2203 x ICPL 20116 (55.8%) and ICPA 2204 x ICPL 20093 (50.1%) with seed yield of 1649, 1604 and 1544 kg/ha respectively. Hybrid ICPA 2203 x ICPL 20116 showed high level of resistance to fusarium wilt and sterility mosaic disease (Table 4). It is clear from the yield data, that obcordate leaf shape of A-lines has no effect on the per se performance of hybrid combinations.

In the present investigation, the long term process of converting already established male sterile lines in to obcordate leaf marker seems to be working with the recovery of some competitive hybrids such as ICPA 2208 x ICPL 20108 (1649.4 kg/ha), ICPA 2203 x ICPL 20116 (1603.9 kg/ha) and ICPA 2204 x ICPL 20093 (1543.8 kg/ha). This is the first time report to use obcordate leaf shape in pigeonpea to track the purity of hybrids and their parental lines. These hybrids will be tested in large scale plots to test their commercial application. The obcordate leaf shape used as NEP is working well and can be use

Table 4. Estimates of standard heterosis (superiority over cultivar Asha) and per se performance for yield and yield contributing traits and disease reaction of crosses.

Hybrids	Days to 50% flowering		Days to 75% maturity		Plant height (cm)		Number of seeds/pod		100-seed mass (g)		Grain yield (kg/ha)		Disease reaction (%)	
	Mean	Heterosis (%)	Mean	Heterosis (%)	Mean	Heterosis (%)	Mean	Heterosis (%)	Mean	Heterosis (%)	Mean	Heterosis (%)	Sterility mosaic	Fusarium wilt
ICPA 2200 X ICPL 20116	116	-4.9	160	-4.9	227.5	0.0	3.9	6.9	10.6	-6.6	1373.3	33.5	0.0	12.5
ICPA 2200 X ICPL 87119	123	0.8	168	0.8	230.0	1.1	3.8	5.6	9.7	-15.0	1170.6	13.7	0.0	12.5
ICPA 2200 X ICPL 20108	115	-5.7 *	158	-5.7*	242.5	6.6	3.8	5.6	10.1	-11.4	1058.1	2.9	0.0	0.0
ICPA 2200 X ICPL 20093	124	1.2	168	1.2	227.5	0.0	4.0	9.7*	10.5	-7.9	888.6	-13.6	0.0	33.3
ICPA 2201 X ICPL 20116	116	-4.9	158	-4.9	235.0	3.3	3.9	8.3	10.6	-6.6	775.3	-24.6	0.0	0.0
ICPA 2201 X ICPL 87119	120	-1.6	165	-1.6	227.5	0.0	3.7	2.8	9.5	-16.3*	786.7	-23.5	0.0	0.0
ICPA 2201 X ICPL 20108	121	-0.8	165	-0.8	242.5	6.6	3.7	2.8	10.0	-11.8	908.6	-11.7	0.0	33.3
ICPA 2201 X ICPL 20093	122	-0.4	168	-0.4	237.5	4.4	3.3	-8.3	9.8	-14.1	644.8	-37.3	0.0	0.0
ICPA 2202 X ICPL 20116	119	-2.9	168	-2.9	245.0	7.7*	3.9	6.9	10.4	-8.8	954.3	-7.2	0.0	0.0
ICPA 2202 X ICPL 87119	121	-0.8	168	-0.8	242.5	6.6	3.9	8.3	10.4	-8.8	980.0	-4.7	0.0	50.0
ICPA 2202 X ICPL 20108	119	-2.9	165	-2.9	240.0	5.5	3.9	6.9	10.0	-12.0	1311.5	27.4	0.0	0.0
ICPA 2202 X ICPL 20093	118	-3.7	163	-3.7	237.5	4.4	4.0	11.1*	9.8	-14.1	683.8	-33.5	0.0	0.0
ICPA 2203 X ICPL 20116	117	-4.5	165	-4.5	227.5	0.0	3.8	4.2	11.0	-3.1	1603.9	55.8	0.0	0.0
ICPA 2203 X ICPL 87119	119	-2.8	170	-2.9	237.5	4.4	3.6	0.0	10.0	-12.3	581.9	-43.4	0.0	0.0
ICPA 2203 X ICPL 20108	119	-2.5	165	-2.5	242.5	6.6	3.6	0.0	10.1	-11.0	1317.1	28.1	0.0	0.0
ICPA 2203 X ICPL 20093	126	2.8	173	2.9	225.0	-1.1	3.9	8.3	9.4	-17.6*	762.9	-25.8	0.0	33.3
ICPA 2204 X ICPL 20116	120	-2.0	168	-2.1	225.0	-1.1	3.7	2.8	11.5	1.3	1455.3	41.5	15.4	7.7
ICPA 2204 X ICPL 87119	120	-1.6	168	-1.6	235.0	3.3	3.9	6.9	10.3	-9.2	1384.8	34.5	10.0	10.0
ICPA 2204 X ICPL 20108	120	-1.6	168	-1.6	235.0	3.3	3.8	4.2	10.3	-9.7	551.5	-46.4	0.0	0.0
ICPA 2204 X ICPL 20093	129	5.3	170	5.3	230.0	1.1	3.8	5.5	10.8	-5.3	1543.8	50.1	0.0	0.0
ICPA 2206 X ICPL 20116	117	-4.1	165	-4.1	235.0	3.3	3.8	5.6	12.6	11.0	1239.1	20.5	0.0	0.0
ICPA 2206 X ICPL 87119	119	-2.9	165	-2.9	232.5	2.2	3.8	5.6	11.1	-2.2	1002.9	-2.5	0.0	23.1
ICPA 2206 X ICPL 20108	124	1.6	170	1.6	235.0	3.3	3.9	6.9	10.9	-4.4	1051.4	2.2	0.0	0.0
ICPA 2206 X ICPL 20093	130	6.6*	175	6.5*	235.0	3.3	3.6	0.0	10.7	-6.5	730.5	-29.0	0.0	0.0
ICPA 2208 X ICPL 20116	118	-3.7	168	-3.7	227.5	0.0	3.9	6.9	10.0	-12.3	997.2	-3.1	45.5	0.0
ICPA 2208 X ICPL 87119	120	-2.1	168	-2.0	240.0	5.5	3.9	6.9	10.2	-10.1	995.3	-3.2	33.3	8.3
ICPA 2208 X ICPL 20108	123	0.4	165	0.4	242.5	6.6	4.0	11.1*	10.8	-5.3	1649.5	60.4	25.0	0.0
ICPA 2208 X ICPL 20093	123	0.8	170	0.8	227.5	0.0	3.7	2.8	10.0	-12.3	858.1	-16.6	0.0	0.0
SE+/-	2.37	-	2.55	-	5.63	-	0.11	-	0.60	-	28.69	-	-	-
CV (%)	2.79	-	2.17	-	3.41	-	4.22	-	8.17	-	213.58	-	-	-

*, ** Significant at 5 and 1 % level of probability, respectively.

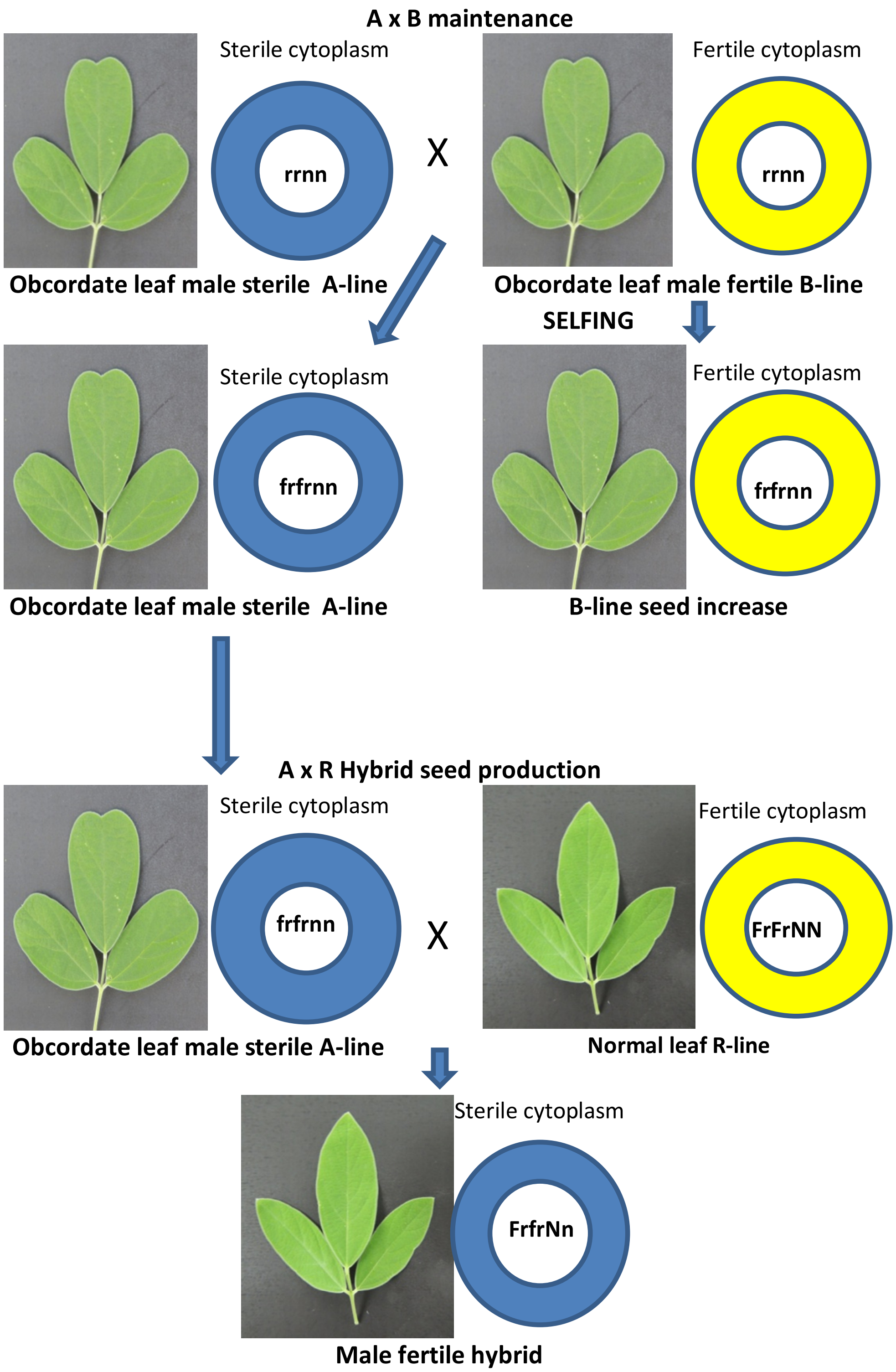

Figure 1. Schematic representation of hybrid seed production activity with obcordate leaf marker.

as a marker both in the maintenance of A-lines and production of F_1 hybrids. The seed production scheme using obcordate leaf marker is presented in Figure 1. When the obcordate leaf male sterile A-line is crossed with the corresponding obcordate leaf male fertile B-line, the resultant progeny will be obcordate leaf male sterile due to the sterile cytoplasm from A-line and presence of obcordate leaf trait in homozygous recessive form. The same seed will be used for A x R hybrid seed production and selfed seed from B-lines will be used for A x B maintenance. The hybrid between obcordate leaf A-line and normal leaf restorer lines will be normal leaf fertile due to complete dominance of normal leaf over obcordate leaf shape and hybrid is fertile due to the interaction of cytoplasmic and nuclear genes.

When farmers buy a seed from any institute or company, they expect to receive a good quality seed. Removing physical impurities like other crops seeds, weed seeds and any inert matter is possible through seed graders and cleaners. The most important factor that deteriorates the actual performance of any hybrid or variety is genetic impurity. In case of hybrids, it is very important to produce and manage the supply of adequate quantities of pure hybrid seeds (Saxena et al., 2010). It is not possible to remove all these admixtures completely with the use of cleaning machines and some seeds which are not true to the type always remain present and it is necessary to conduct purity test or analysis, to determine percent admixture present in the seed lot. However, it is traditionally done with grow out test but not practically and economically feasible due to long growing season of pigeonpea crop. The main idea with this technology is, when a commercial seed lot is received for seed purity assessment from different production sources, we can use a representative sample from each lot to undertake grow out test to check the purity of hybrid seed. It takes around 4 to 6 weeks after sowing to express the obcordate leaf shape which is a very short period as compared to regular grow out test in which we have to wait for six months or more. Since normal leaf shape is completely dominant over obcordate leaf shape, the pure hybrid will have a normal leaf seedling. The off types in the A-line maintenance can be identified with any normal leaf seedling and if hybrids showed obcordate leaf shape seedlings, they can be easily discarded for further seed distribution.

Conflict of Interest

The authors have not declared any conflict of interest.

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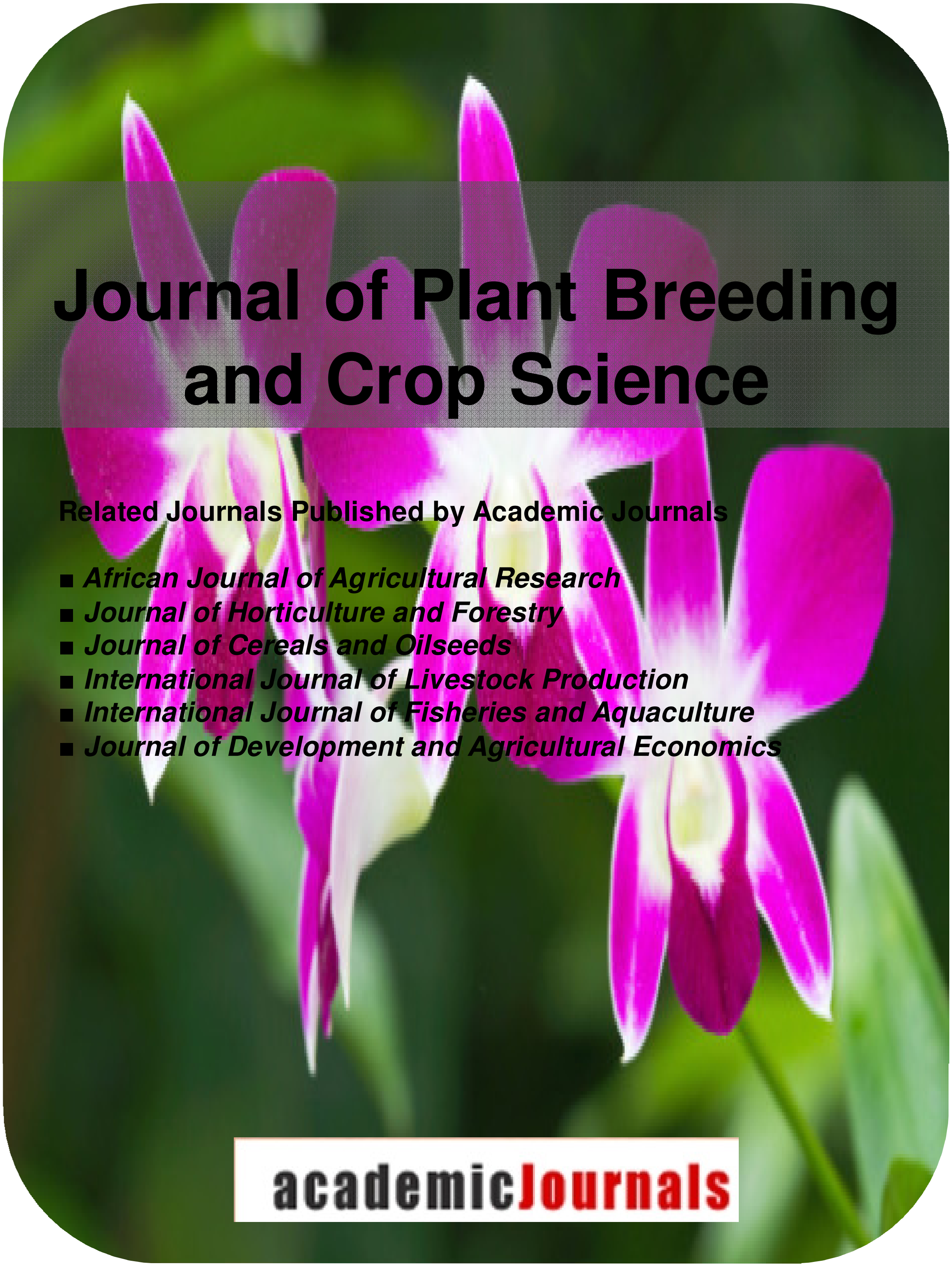

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