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Research Article

Simple, Rapid And Cost Effective Screening Method For Drought Resistant Breeding In Pearl Millet

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Abstract
Pearl millet is one of the most important cereals grown in drought-prone areas and is the staple grain for million of people in West Africa and India. Breeding for drought-prone environments is constrained by lack of suitable selection indices of drought stress resistance. The present study is conducted to determine the reliability of *in vitro* screening method for initiating drought breeding programme. This *in vitro* screening method proves to be an ideal method for screening large set of germplasm with less efforts accurately and cost effective. This experiment was carried out with a collection of twenty one millet genotypes including commercial varieties and advance hybrid cultures tested in completely randomized design. Data were recorded at five different moisture stress levels (-3, -5, -7.5, -10 bars and control) by using polyethylene glycol (PEG) 6000 on germination percentage, root length, shoot length, root / shoot ratio and statistically analyzed for significant differences. The genotypes recorded significant differences for all traits in response to various moisture stresses. The genotype TNBH 0538 gave the good germination percentage, root length, shoot length, and root/shoot ratio as compared with commercial cultivars under all five moisture stresses. ICMV- 221 showed highest resistance against moisture stress, while PT6034 showed lowest resistance. TNBH 0642 also gave the better performance under all four moisture levels for most of the traits at seedling stage. The regression studies indicated, the osmotic stress were the most suitable method for drought tolerance screening owing to their highly significant relationship with declining root length (R^2 = 0.991; $P < 0.001$) and shoot length (R^2 = 0.998; $P < 0.001$). Hence, the system used in this study appeared to be a simple, rapid and cost effective method for screening seedling traits response to water stress condition to improve the drought tolerance in pearl millet.

Key words: Osmotic stress, Bars, PEG and Drought Resistance

Introduction

India is the largest producer of pearl millet (*Pennisetum glaucum* (L.) R. Br.) and occupies fourth position among the cereals next to the rice, wheat and sorghum, both in terms of area (9.43 mha) and production (8.01 mt), with an average productivity of 850 kg ha^{-1} (Khairwal 2007). It is the principle food crop across sub Saharan Africa and north western India, but in terms of world production, pearl millet is not a major cereal. Although demand for pearl millet grain as human food in India is currently decreasing, it is emerging as forerunner in the form of alternative food, feed and industrial products. Being a C4 species, it has tremendous potential for biomass production, most of which is accumulated in its vegetative parts (Appa Rao 1999). There is a new interest in USA in growing pearl millet as grain crop because of its drought tolerance and high quality grain (Andrews *et al*., 1993). Pearl millet is most droughts tolerant of all domesticated cereals and soon after its domestication it became widely distributed across the semi arid tropics of Africa and Asia. The crop raised under traditional rainfed farming method with most of the production being centred in drier marginal area of less than 500 mm of annual rain fall. However, the productivity in arid zone is lower due to low and erratic rainfall is the single most important constrain to millet production, hence, the need for breeding drought tolerant early maturing varieties for the better food security where the millet grown as a stable food crop.

Breeders and farmers aim to get higher seedling establishment in crops, but some biotic and abiotic stresses reduce seedling establishment in field conditions. Recently, salt and drought stress are perhaps the two most important abiotic stresses that limit plant growth and development. These abiotic stresses occur in field condition due to lack of some

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environmental components. The uncertainty of rainfall is immediately after plant emergence, leading to early season drought in rainfed farming systems (El Hafid *et al*., 1998).

One of the greatest challenges in drought is to sow a seed type that has the capacity to produce abundant biomass and cover in a short period of time (Van den Berg, 2002). Pearl millet is one of those cereal grasses which has strong development of underground organs and tends to have efficient adaptive mechanisms to cope with drought (Winkel and Do, 1992; Winkel *et al*., 1997; Bezançon *et al*., 1997). Because of its tolerance to high temperature and better ability to withstand drought and to grow even in low soil fertility conditions, pearl millet is best suited for arid and semi-arid regions of the country (Khairwal *et al*., 2007).

The study of the influence of the drought using osmotic solutions is one of the methods in the study of resistance during the germinal phase. Several literatures have indicated the superiority of the germination capacity of pearl millet compared to that of sorghum as well as its remarkable resistance in the drought (Van Den Abeele and Vandenput, 1956; Gaudy, 1957; White and Cooper., 1959; Martin and Leonard, 1967). Radhouane (2007) studied seeds of pearl millet from six provenances of Tunisia were subjected to germination and shoot and root length traits on filter paper treated with polyethylene glycol 6000 (PEG 6000) solutions and found PEG useful for early screening . Early and rapid elongation of roots is important indication of drought resistance. Ability of continued elongation of root under situation of water stress was remarkable character of most crop species and root length is more affected to drought condition than shoot length (Kulkarni and Deshpande, 2007). With this background, the present study was aimed to assess the effect of polyethylene glycol on root and shoot trait in seedling of pearl millet population and to identifying the superior genotypes for drought tolerance.

MATERIALS AND METHODS

Genetic materials

Experimental material comprised of twenty one different pearl millet genotypes having broad genetic base, newly developed hybrid (under advance yield trials) and commercial cultivars varied by date of release, pedigree, and yield as well as quality traits (based on the earlier report, data not shown). The popular commercial cultivars and hybrids included in this study are X7, Co 7, Co (Cu) 9 and ICMV 221 used as check variety, which was bred by random mating of 124 selected S_1 progenies of Bold Seeded Early Composite (BSEC) drought trial.

Action of polyethylene glycol

Osmotic solutions are used to impose water stress reproducibly under *in vitro* conditions (Pandey and Agarwal, 1998). Polyethylene glycol molecules with a Mr $\geq$ 6000 (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Hohl and Schopfer, 1991; Lu and Neumann, 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita *et al.,* 1979; Saint-Clair, 1976). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low molecular osmotica, which infiltrate the cell wall with solutes (Veslues *et al*., 1998).

Laboratory experiment

The experiments were conducted on twenty one pearl millet genotypes, which included twelve inbreds, five hybrid culture and four commercial cultivars during the June-July,2008 in Cytogenetics Laboratory of Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Water stress was applied through four concentration of PEG (6000 MW), with -3.0, -5.0, -7.5 and -10.0 bars of water potential prepared by dissolving 115, 196, 235 and 289 grams respectively in 1000 ml of distilled water following the method of Hadas (1976) . Fifty randomly selected seeds of each pearl millet genotype were placed on the moistened germination paper according to Bayoumi *et al*., (2008) to provide appropriate moisture stress for seed germination. When seedlings were at stage of first true leaf initiation (10 days after treatment) data were recorded at four different moisture levels on germination percentage, root length, shoot length and root / shoot ratio. The experiment was designed as a completely randomized design with two factors. The first factor was the genotypes and the second one is the external water stress treatments. Data were analysed with ANOVA, and means were separated by an LSD using $P < 0.05$. All the analyses were done by using GenStat 12th edition Statistical software (VSN International Ltd, 2009).

Results And Discussion

Significant differences were observed under different PEG concentration (0.0 (control),-3.0, -5.0, -7.5 and -10.0 bars) for all the characters under the study (Table 1).

Effect of PEG on Germination percentage

Water deficit in soil affects the germination of seed and the further growth of seedlings negatively. Different levels of water stress induced by PEG had significant effects on the seed germination (Table 1). The utmost germination (96 %) in TNBH 0538, TNBH 0642, Co 7 and Co (Cu) 9 and the minimum germination capacity in P.T. 5188 and P.T. 6034 (68 %) in control (0.0 bar) was observed. The germination capacity significantly varied (48.5 % in -10.0 bars to 86.86 % in control) between the treatments than the genotypic differences. Results of the current study were in agreement with Radhouane (2007) in pearl millet, Farsiani and Ghobadi (2009) in corn, Van den Berg and Zeng (2006) in grass species, Kalefetoglu *et al.* (2009) in chickpea, Almansouri *et al*. (2001) and Soltani *et al,* (2006) in wheat. Among the genotypes tested, ICMV-221, Co 7, TNBH 0538 and X 7 had shown better germination potential than others. Moreover, P.T. 6034 and P.T. 5541 were most affected by an external water stress of -10.0 bars with mean germination percentage of 48% and 54.4 % respectively and the check variety ICMV 221 had highest germination in most of the concentration. The final germination percentage varied between 96 % (TNBH 0538, 0642, Co 7 and Co (Cu) 9) for the control and 20 % (P.T. 6034) for the most concentration of PEG solution. It was observed that germination percentage with decreasing water potential of the environment, probably was caused by the low hydraulic conductivity of the environment, where PEG 6000 makes water unavailable to seeds, affecting the imbibitions process of the seed which is fundamental for germination (Lobato *et al*, 2008). The lowest germination percentage was observed at -10.0 bars (Table 1). This larger reduction with PEG solution could be attributed to high viscosity, where solubility and diffusion of oxygen were reduced compared to control (Delachiave and De Pinho, 2003). At water potential -10.0 bars, the rate of germination of the pearl millet population was below 50% which is similar to the results obtained by Singh and Singh (1981a, b, c; Singh 1983; Hadas and Stibbe, 1973).

Effect of PEG on root length

Early and rapid elongation of root is important indication of drought tolerance. A root system with longer root length at deeper layer is useful in extracting water in upland conditions (Kim *et al*, 2001; Narayan, 1991). In the present investigation, the root length also significantly declined with increased external water potential (Table 1, Fig 2) and consequently, all treatments caused a decrease in root elongation in all genotypes compared to their controls. The mean root length varied from 6.9 cm (P.T.5554) to 27.3 cm (Co (Cu) 9). In all the stress conditions, it was observed that, genotype TNBH 0642 showed higher root mean length (21.52 cm) and also shown least retardation of root length in different water potential conditions. At higher concentration of PEG (-10.0 bar), the genotype TNBH 0503 and P.T.6017 recorded highest root length 20.3 cm and 18 cm respectively. The genotype P.T. 5554 was distinguished from the other population by its reduced root length (< 7 cm). Comparable results have been reported by various authors, Walter (1963), Parmer and Moore (1966) Radhouane (2007) and Kulkarni and Desphpande (2007). The regression studies indicated, the osmotic stress were the most suitable method for drought tolerance screening owing to their highly significant relationship with declining root length ($R^2 = 0.991$; $P < 0.001$) (Fig 2).

Effect of PEG on shoot length

Root length is more affected to drought condition than shoot length, but the symptoms/ effect of drought exhibited mostly on the shoot as well as aerial parts of the plant, which will bear most economic parts of field crops in field conditions. Hence, the shoot parameters will also helping the breeder while selecting the superior genotypes against drought. In the present study, the shoot length was decreased with an increasing in external water stress (Fig 2). However, this retardation was found to be high in P.T. 5554 (2.80 cm) at high concentration of PEG and low in Co 7, TNBH 0642 and TNBH 0508 with the shoot length of 8.30, 8.05 and 8.02 cm, respectively. These genotypes also showed highest individual mean shoot length of 10.02, 9.68 and 9.70 cm in all the treatment conducted in the laboratory. Lawlor (1969) have also been observed the retardation growth of shoot and root length in response to increasing moisture stress under field as well as laboratory condition. Our results were similar to earlier studies (Walter 1963; Parmer and Moore 1966; Radhouane, 2007; and Kulkarni and Desphpande, 2007). It is generally accepted that the roots suffer first from exposure to stresses, followed by their associated plant parts (Misra and Dwivedi, 2004). The regression analyses revealed that, osmotic stress were the most suitable method for drought tolerance screening owing to their highly significant relationship with declining the shoot length $R^2 = 0.998$, $p < 0.001$) (Fig 2) indicating that, the drought stress induced by PEG had inhibited shoot elongation at higher rate than the root growth and shoot / root ratio. Similar result was also reported by Kalefetogllu et al., (2009).

Effect of PEG on root / shoot ratio

Apart from the root length and shoot length, root / shoot ratio also plays a major role in selecting the line for drought tolerance and balanced root and shoot growth was observed in drought resistant genotypes. Entries selected for high root/shoot ratio demonstrated significantly improved drought tolerance compared to their parents, whereas improved drought tolerance for field-selected entries was less consistent.The present study revealed significant variations for the root / shoot ratio among the population. The ratio ranged from 1.2 (P.T. 5188) to 3.5 (TNBH 0545) in the control. The highest root/shoot was ratio observed in TNBH 0503 (3.90) and lowest root/ shoot ratio of 1.60 observed in two genotype *viz*., P.T. 6029 and P.T. 6040.

Simple effective method for early drought screening

Drought tolerance screening under field condition involves lot of resources (land, men power) and the environmental influences that affects phenotypic expression of genotype. The *in vitro* screening method proves to be an ideal method to screen large set of germplasm with less effort, accurately and the growth pattern differences are due to genotypes with least environmental influences. The germination of seed under simulated drought conditions offers possibilities for revealing seed weaknesses and predicting relative differences among seed lots in field emergence. However, these experiment to be additionally supported by field evaluation methods to validate drought resistant genotypes. Field screening requires full season field data and it's not always convenient or efficient, hence need to have simple and effective early screening method (Kim *et al*., 2001).

The use of PEG for the experimental control of external water potential has been proved to be very effective method for studying the effect of water stress on seed germination and seedling growth characters (Hadas, 1976; Aquila *et al*., 1984; Kim *et al*., 2001; Van den Berg and Zeng, 2006; Radhouane, 2009) and simple cost effective method to screen large set of germplasm within very less time period and accurately (Kim *et al*., 2001; Kulkarni and Deshpande, 2007). On the basis of present investigation, imply that this technique can be adopted for pearl millet as well other crops for early drought screening for fast track drought breeding programme.

Results of this study revealed that severe drought (PEG-induced) stress can negatively affect germination percentage, followed shoot and root length. It has been reported that PEG-induced osmotic stress can cause hydrolysis of storage compounds that further lower the internal osmotic potentials of the seed (Hampson and Simpson, 1990). All the genotypes showed significant differences at various concentrations, but the genotypes PT 6034, PT 5541, PT 5554 were most affected than others, suggesting that, most of the genotypes of pearl millet could germinate even in the presence of a potential of -10.0 bar. Nevertheless, the regression analyses revealed the osmotic (PEG) stress were the most suitable method for drought tolerance screening owing to their highly significant relationship with declining the germination percentage, root and shoot length. Hence, the system used in this study appeared would be a simple, rapid and cost effective method for screening seedling traits response to water stress condition.

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Table 1. Analysis of variance for germination and growth characters of twenty one pearl millet genotype during drought induced by polyethylene glycol

Source	df	Mean square			
		Germination %	Root length (cm)	Shoot length (cm)	Root / shoot ratio
Genotype (G)	20	1196.05**	167.877**	54.826**	3.956**
Treatment (T)	4	16269.72**	576.454**	159.080**	0.536**
G x T	80	119.33**	14.44**	2.571**	0.345**
Error	210	3.761	0.223	0.047	0.004

** Significant at 1 % probability levels

Figure 2. Relationship between PEG concentration (bars) with seedling root length and shoot length (cm)

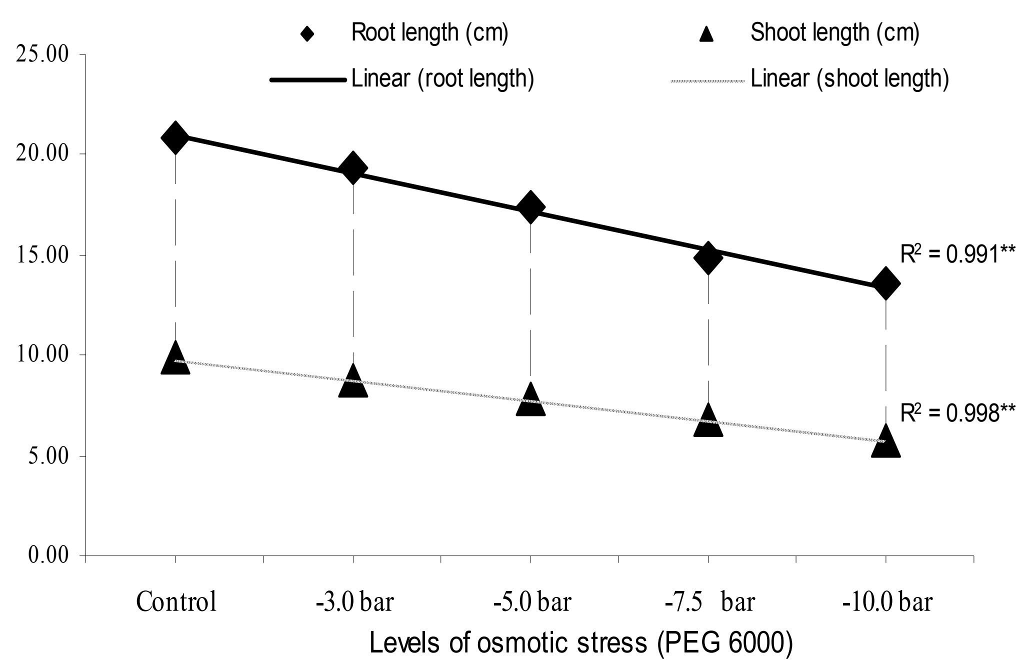

Figure 1. Germination percentage of pearl millet seeds exposed to different osmotic potentials induced by PEG 6000

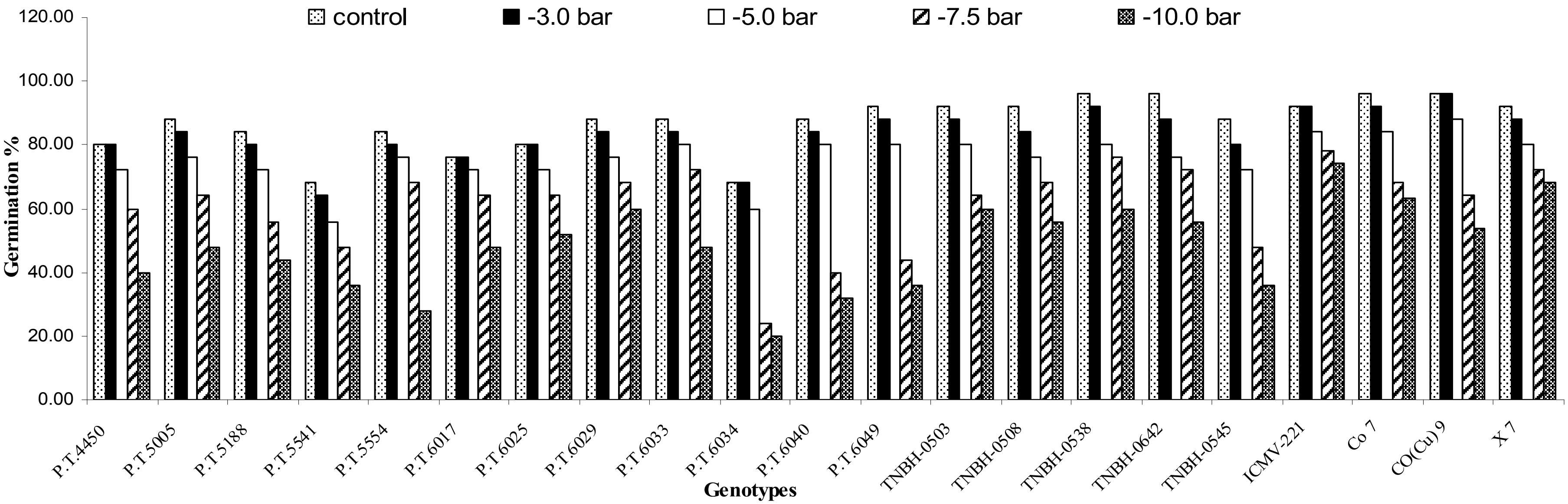

Table 2. Effect of osmotic stress on seedling traits of peal millet genotypes during drought induced by polyethylene glycol *in vitro* condition

Genotypes	Germination %					Root length					Shoot length					Root / Shoot ratio				
	External water potential (bars)					External water potential (bars)					External water potential (bars)					External water potential (bars)				
	Control	-3	-5	-7.5	-10	Control	-3	-5	-7.5	-10	Control	-3	-5	-7.5	-10	Control	-3	-5	-7.5	-10
P.T.4450	80.0	80.0	72.0	60.0	40.0	20.3	18.3	16.5	14.0	13.3	6.0	5.2	5.0	4.4	3.8	3.4	3.5	3.3	3.2	3.5
P.T.5005	88.0	84.0	76.0	64.0	48.0	15.9	17.3	17.0	15.2	15.0	8.4	7.1	6.6	6.0	5.9	1.9	2.5	2.6	2.5	2.5
P.T.5188	84.0	80.0	72.0	56.0	44.0	13.6	17.4	15.4	15.5	14.8	11.7	8.9	7.8	6.9	5.9	1.2	2.0	2.0	2.2	2.5
P.T.5541	68.0	64.0	56.0	48.0	36.0	20.8	18.1	18.0	17.0	13.9	5.9	5.8	5.4	4.9	4.6	3.5	3.1	3.4	3.5	3.0
P.T.5554	84.0	80.0	76.0	68.0	28.0	10.3	6.7	8.2	7.1	6.9	6.6	5.3	5.3	3.7	2.8	1.6	1.3	1.5	1.9	2.5
P.T.6017	76.0	76.0	72.0	64.0	48.0	24.3	23.5	19.2	19.5	18.0	10.9	10.5	9.3	8.0	5.9	2.2	2.2	2.1	2.4	3.0
P.T.6025	80.0	80.0	72.0	64.0	52.0	24.5	20.7	18.7	16.3	15.9	9.3	8.0	7.7	7.7	6.8	2.7	2.6	2.4	2.1	2.3
P.T.6029	88.0	84.0	76.0	68.0	60.0	20.5	20.8	17.8	13.6	12.1	11.0	9.1	9.3	8.6	7.6	1.9	2.3	1.9	1.6	1.6
P.T.6033	88.0	84.0	80.0	72.0	48.0	18.0	17.7	17.5	16.1	11.9	7.1	5.9	5.4	5.2	3.4	2.5	3.0	3.2	3.1	3.5
P.T.6034	68.0	68.0	60.0	24.0	20.0	15.1	11.4	10.2	9.5	8.6	6.2	5.8	5.8	4.6	3.9	2.4	2.0	1.8	2.1	2.2
P.T.6040	88.0	84.0	80.0	40.0	32.0	19.0	18.6	17.6	12.7	8.0	13.7	13.3	9.6	5.8	5.0	1.4	1.4	1.8	2.2	1.6
P.T.6049	92.0	88.0	80.0	44.0	36.0	20.4	15.9	13.9	12.4	12.0	10.7	8.7	8.5	7.6	6.4	1.9	1.8	1.6	1.6	1.9
TNBH-0503	92.0	88.0	80.0	64.0	60.0	17.6	24.4	23.5	11.3	20.3	10.7	8.9	7.9	7.1	5.2	1.7	2.8	3.0	1.6	3.9
TNBH-0508	92.0	84.0	76.0	68.0	56.0	26.6	24.5	19.4	17.9	14.7	11.6	10.8	9.1	9.0	8.0	2.3	2.3	2.1	2.0	1.8
TNBH-0538	96.0	92.0	80.0	76.0	60.0	23.0	22.1	21.1	18.6	15.6	12.9	10.9	9.6	8.2	6.6	1.8	2.0	2.2	2.3	2.4
TNBH-0642	96.0	88.0	76.0	72.0	56.0	25.8	23.2	22.7	19.6	16.3	11.2	10.9	9.3	9.0	8.1	2.3	2.1	2.4	2.2	2.0
TNBH-0545	88.0	80.0	72.0	48.0	36.0	20.9	19.1	15.2	12.7	11.3	6.0	5.9	5.1	4.5	3.8	3.5	3.2	3.0	2.8	3.0
ICMV-221	92.0	92.0	84.0	78.2	74.0	25.2	18.4	16.5	15.6	13.0	11.6	10.8	9.8	8.1	7.2	2.2	1.7	1.7	1.9	1.8
Co 7	96.0	92.0	84.0	68.0	63.0	24.3	21.8	20.4	18.9	16.3	11.0	10.7	10.1	10.0	8.3	2.2	2.0	2.0	1.9	2.0
CO(Cu) 9	96.0	96.0	88.0	64.0	54.0	27.3	23.7	17.5	12.3	10.8	11.8	10.8	9.9	6.5	4.9	2.3	2.2	1.8	1.9	2.2
X 7	92.0	88.0	80.0	72.0	68.0	24.0	23.3	18.7	16.5	15.9	12.9	9.8	8.6	8.0	7.2	1.9	2.4	2.2	2.1	2.2
Trt. Mean	86.9	83.4	75.8	61.1	48.5	20.8	19.4	17.4	14.9	13.6	9.9	8.7	7.8	6.9	5.8	2.2	2.3	2.3	2.2	2.5
S. E Genotype (G)	0.708					0.172					0.079					0.023				

Treatment (T)	0.346	0.084	0.039	0.011
G x T	1.583	0.385	0.177	0.051
LSD (p<0.05)				
Genotype (G)	1.396	0.34	0.156	0.045
Treatment (T)	0.681	0.166	0.076	0.022
G x T	3.122	0.759	0.349	0.101