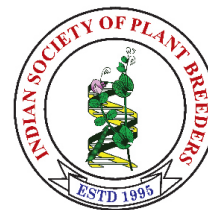# Electronic Journal of Plant Breeding

Research Article

Assessment of drought tolerance in Nagina 22 mutants of rice using Poly Ethylene Glycol (PEG)

K. Manonmani[1*], P. Jeyaprakash[2], S. Manonmani[1], M. Raveendran[3] and P. Jeyakumar[4]

[1]Department of Genetics and Plant Breeding,
[2]Department of Plant Breeding and Genetics, Anbil Dharmalingam Agricultural College and Research Institute, Trichy – 620 009, Tamil Nadu, India.
[3]Department of Plant Biotechnology,
[4]Department of Plant Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
*E-Mail: dr.manoagri1314@gmail.com

Abstract
A laboratory experiment was conducted to screen 500 EMS induced mutants of Nagina22 using low molecular osmotic PEG-6000. The analysis of variance showed that all ten characters showed significant variation at 1% probability level. Nagina22 was screened under six moisture regimes as 0, -2, -4, -6, -8 and -10 bars of PEG-6000 and -6 bars was fixed as optimum concentration for mutant screening. Results of correlation revealed that relative seedling vigour showed positive significant correlation with all characters studied except root length stress index and relative root number. Based on PCA, emergence percentage, promptness index and seedling vigour was considered as selection criteria for selecting drought tolerant mutants under moisture stress. N22-PDT-17 and N22-PDT-64 recorded highest value for all the characters studied except number of roots than Nagina22. Hence, these two mutants were selected as better mutants with altered drought response and could be further validated under natural drought environment.

Keywords
Drought, Nagina22, Mutant, Polyethylene glycol

INTRODUCTION

Rice is the staple food for almost half of the world's population and according to changing environment, there is a need to increase the production to feed growing population. Drought is the major abiotic stress which hampers the growth and production, and reduces the harvest size. Uneven distribution of rainfall as well as reduction in groundwater table leads to development of drought tolerant varieties (Pandey and Sukla, 2015). But breeding for drought tolerance is very complicated. Yield loss mainly depends on stage, duration and severity of drought (Sokoto and Muhammad, 2014). Apart from active tillering, booting and grain filling stage, two stages *i.e.,* germination and early seedling growth are the most critical stages drastically affected by drought stress.

Drought screening for large number of entries is comparatively easy under *in vitro* condition than in field condition. While using polyethylene glycol, mannitol or other low molecular weight osmotic, there is an increase in drought stress as well as steep decline in moisture content of tissue. The mode of action of PEG is that it will withdraw water not only from cell but also from cell wall and also affects the moisture availability in the germination medium (Gharoobi *et al.,* 2012). Among the low molecular osmotica, PEG mimics a way which is simil ar to soil under moisture stress situation and hence, it has been used to select drought tolerant genotypes under laboratory condition. The aim of this work was to study the comparative effect of different concentrations of polyethylene glycol on germination and growth characteristics and also to identify the mutants with better drought response than Nagina 22. To support the above research, correlation was made to identify the cause and effect relationship between variables and

also principal component analysis to identify the principal component contributing towards maximum % variation in mutants.

MATERIALS AND METHODS

A large set of Ethyl Methane Sulphonate (EMS) induced mutants in the upland rice variety Nagina22 was developed through a National Initiative involving six Research Institutes. M_1 to M_3 generations were raised at the Department of Rice, Tamil Nadu Agricultural University, Coimbatore. Drought screening of 500 Nagina22 M_4 generation mutants were done using polyethylene glycol under laboratory condition. The experiment was carried out in Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore during December, 2018 to August, 2019.

Ten seeds of each mutant were surface sterilized with 70% ethanol for five minutes. The seeds were then washed three times with sterile distilled water to remove ethanol. Sterile water was used to avoid contamination. Seeds were evenly distributed *i.e.,* approx. 10 cm gap between seeds in sterile petri dish. Each petri dish was filled with 15 ml of distilled water or polyethylene glycol (PEG-6000: Sigma Chemicals) to resemble drought stress.

a) Standardization of PEG-6000 concentration:

The experiment was laid out in completely randomized design (CRD) with sixdifferent concentrations and three replications each. Distilled water was used as control (0 bars) and osmotic potentials -2 bars, -4 bars, -6 bars, -8 bars and -10 bars were created by addition of 12.80 g, 18.90 g, 23.50 g, 27.40 and 30.95 g PEG-6000 in 100 ml of distilled water respectively.

b) Screening of Nagina 22 mutants:

The experiment was laid out in completely randomized design (CRD) with 500 mutants in two replications for both control and drought.

1. Emergence percentage % (EP)

When both the plumule as well as radical extended to more than 2 mm, it was considered a germinated one. Every 24 hours interval, seed germination was recorded.

$$\text{Emergence percentage} = \frac{\text{No. of germinated seeds}}{\text{Total no of seeds}} \times 100$$

2. Promptness index (PI)

Promptness index is the percentage of seeds germinated on first four even days *viz.,* 2nd, 4th, 6th and 8th day and which is denoted as nd2, nd4, nd6 and nd8, respectively (Ashraf and Mehmood, 1990).

$$\text{PI} = nd2(1.00) + nd4(0.75) + nd6(0.5) + nd8(0.25)$$

Where nd2 = 2nd day, nd4 = 4th day, nd6 = 6th day, nd8 = 8th day

3. Germination index % (GI) and Germination stress tolerance index % (GSTI)

Germination index and Germination stress tolerance index was calculated based on stressed and normal condition (Ashraf and Mehmood, 1990).

$$(GI) = \frac{\text{Germination \% under stress}}{\text{Germination \% under control}} \times 100 \qquad GSTI (\%) = \frac{\text{PI under stress}}{\text{PI under control}} \times 100$$

4. Plant height stress index % (PHSI) and Root length stress index % (RLSI)

Plant height and root length were measured on 14th day (Ellis and Roberts, 1981).

$$RLSI = \frac{\text{Plant height under stress}}{\text{Plant height under control}} \times 100 \qquad PHSI (\%) = \frac{\text{Root length under stress}}{\text{Root length under control}} \times 100$$

5. Seed vigour (SV) and Relative seedling vigour % (RSV)

Seed vigour was calculated based on germination percentage and seedling length (Ellis and Roberts, 1981). Relative seedling vigour was calculated based on seedling vigour at stress and control condition.

$$SV = \text{Germination percentage} \times \text{Seedling length}$$

$$RSV = \frac{\text{Seedling vigour under stress}}{\text{Seedling vigour under control}} \times 100$$

6. Relative seedling weight % (RSW) and Relative number of roots % (RR)

Seedling weight was calculated in terms of milligram on 14th day. Relative seedling weight and relative number of roots calculated based on seedling weight and number of roots at stress and control condition, respectively.

$$RSW = \frac{\text{Seedling weight under stress}}{\text{Seedling weight under control}} \times 100 \qquad RR = \frac{\text{Number of roots under stress}}{\text{Number of roots under control}} \times 100$$

7. Drought susceptibility score

According to Standard Evaluation System for rice (IRRI, 1980), 0-9 visual scores for stressed plants was recorded. The mutants which showed lower score were considered as drought tolerant mutant and vice versa.

The data were collected and subjected to analysis of variance, principal component analysis and correlation using Minitab v16.0 and SPSS v16.0.

RESULTS AND DISCUSSION

The results of analysis of variance (ANOVA) showed that all the ten characters namely, emergence percentage, promptness index, germination stress tolerance index, plant height stress index, root length stress index, germination index, relative seedling weight, relative

number of roots, visual drought susceptibility score and relative seedling vigour exhibited significant difference between control and different concentration of PEG-6000 *viz.,* -2 bars, -4 bars, -6 bars, -8 bars and -10 bars at 1% probability level (Table 1).

The mean values of germination and seedling characters of Nagina22 on different drought stress were compared by means of Duncan's multiple range test (DMRT) (Fig. 1). The mean value followed by different letters (a,b,c,d) in each column showed that there was a significant

difference observed between different PEG-6000 concentrations on the respective characters (Table 2). Based on the performance of Nagina22 on different concentration of moisture stress, -6 bars was fixed as optimum concentration for screening 500 mutants. The mean, minimum and maximum values of 500 EMS induced mutants were presented in Table 3 and their histograms are presented in fig. 2. The best performing mutants at -6 bars were considered as drought tolerant mutant or altered drought response than Nagina22 and it is presented in fig. 3.

Table 1. Analysis of variance on mean sum of squares over different treatment of drought stress in Nagina22

Sources of variation	df	EP	PI	GSTI	PHSI	RLSI	GI	RSW	RR	Score	RSV
Treatment	4	2115.0**	295.7**	3171.5**	3028.8**	6604.4**	2115.0**	1501.8**	1494.9**	16.1**	3087.9**
Error	9	20.0	0.6	1.9	3.9	21.4	20.0	12.3	1.3	0.4	0.6
C.V(%)		7.71	2.83	2.88	3.40	5.02	7.71	7.20	2.12	14.63	1.72

(df – degrees of freedom, CV – Coefficient of Variance, ** Significant at 1% probability, * Significant at 5% probability)

Table 2. Mean comparison of different drought stress levels in Nagina22 using Duncan's multiple range test (DMRT)

Treatments	EP	PI	GSTI	PHSI	RLSI	GI	RSW	RR	Score	RSV
-2 bars	90.00a	7.63a	93.88a	95.70a	157.00a	90.00a	76.40a	92.80a	0.85b	87.80a
-4 bars	70.00b	5.88ab	70.82b	93.85a	118.30b	75.00a	65.55a	62.95b	2.00ab	77.65a
-6 bars	70.00b	5.63ab	70.94b	65.35b	117.00b	75.00a	61.30a	56.50b	5.00ab	52.10b
-8 bars	40.00c	1.75ab	18.13c	23.50c	55.30c	40.00b	29.90b	27.90c	7.00a	8.80c
-10 bars	10.00d	0.38b	2.69c	12.10c	12.50d	10.00c	10.75c	27.20c	7.00a	1.20c
SE	4.18	1.48	5.43	5.11	5.99	4.27	3.92	3.74	1.36	5.82

(Meanfollowed by different letters within column showed significant difference @ P< 0.05 by means of DMRT, EP-Elongation percentage, PI- Promptness index, GSTI – Germination stress tolerance index, PHSI – Plant height stress index, RLSI – Root length stress index, GI – Germination index, RSW – Relative seedling weight, RR – Relative number of roots, Score – Visual score, RSV – Relative seedling vigour)

Table 3. Mean performance of 500 EMS induced mutants of Nagina22 under drought stress

Traits	EP	PI	GSTI	PHSI	RLSI	GI	RSW	RSV	RR	Score
Nagina22	85.0	5.0	70.9	44.8	63.6	85.0	69.0	38.1	90.5	5.0
Mean	64.7	3.8	47.6	40.4	101.8	67.9	52.7	27.6	66.8	6.5
Min	15.0	0.5	5.7	3.7	11.7	15.6	10.3	2.2	37.1	3.0
Max	100.0	7.4	123.1	130.6	397.9	175.0	139.3	133.0	183.4	9.0
Variance	303.5	1.7	297.9	520.4	3318.8	386.5	557.2	347.3	214.6	1.1
SD	17.4	1.3	17.3	22.8	57.6	19.7	23.6	18.6	14.6	1.1
C.V (%)	26.9	34.3	36.3	56.5	56.6	28.9	44.8	67.6	21.9	16.5

(Min – Minimum, Max – Maximum, SD – Standard deviation, C.V. – Coefficient of variance)

Emergence percentage was considered as criteria for selecting better performing mutant in terms of drought tolerance. Emergence percentage was decreased with increased PEG-6000 concentration. Among 500 mutants studied, four mutants *i.e.,* N22-PDT-28, N22-PDT-68, N22-PDT-155 and N22-PDT-254 recorded 100% emergence, which is similar to control. Thirty mutants had 90-95% emergence and 46 mutants had 85% emergence. Good emergence percentage might reflect the potentiality

of crop at remaining growth stages and in mean while, those entries were expected to produce more yield. The similar results were reported by Govindaraj *et al.,* 2010 and Petrovic *et al.,* 2016. Germination index is inversely proportional to moisture stress. Nagina22 had germination index of about 85%. Among the mutants studied, 47 mutants had higher germination index than Nagina22. The mutants which excelled their performance in emergence percentage showed good value in GI. Severe drought

stress always slows down the seeds water uptake and thereby stops better seed germination and the result was in accordance with the findings of Islam *et al.,* 2018.

Drought not only affects germination, but also affects the mean germination time. Under higher drought, there was a delay in seedling emergence rate due to reduction in cell division as well as metabolism. Promptness index was calculated based on emergence percentage at 2nd, 4th, 6th and 8th day of stress. Among the mutants screened, three mutants *viz.,* N22-PDT-3 (7.25), N22-PDT-25 (7.00) and N22-PDT-38 (7.38) had PI value more than 7.00. As

a whole, 85 mutants had PI value higher than Nagina22. It showed that these mutants were able to emerge evenly at water deficit condition.

Germination stress tolerance index was mainly used to identify the differences in rate of germination under osmotic stress as well as control. Among mutants, 38 mutants had higher GSTI than Nagina22. Three mutants had GSTI more than 100%. It showed that there was higher PI recorded in stress condition than control. This may be because of poor quality seeds. The similar result was reported by Taiz and Zeiger, 2006.
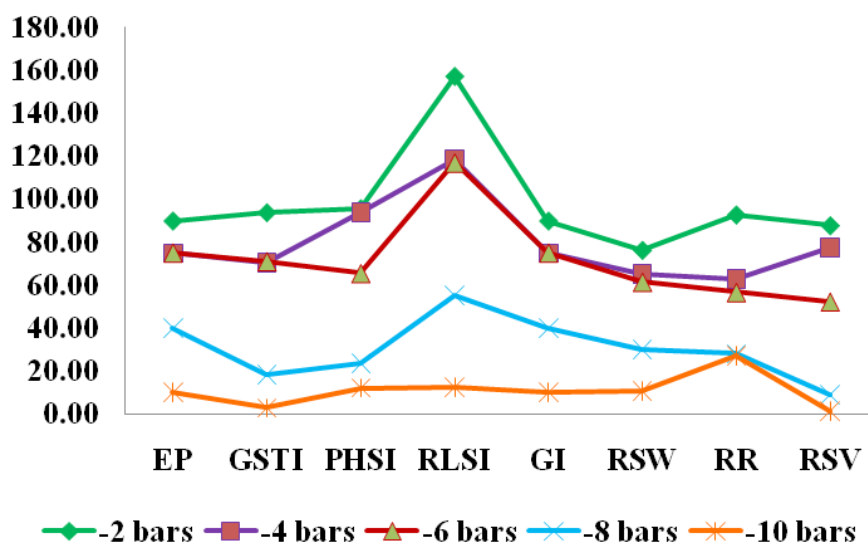
Fig. 1. Mean comparison of different seedling traits and indices of Nagina22 with five different levels of PEG-6000

Plant height stress index was calculated based on the seedling length under control and drought stress. Among the mutants, 177 mutants had higher PHSI than Nagina22. The minimum PHSI was recorded by N22-PDT-281 (3.7) and the maximum was recorded by N22-PDT-208 (130.6). Relative seedling weight varies with seedling length, root length and number of roots. RSW decreased with increased PEG-6000 concentration. Among the mutants studied, 119 mutants had higher seedling weight than Nagina22. Initial seedling weight produces high biomass and it tends to have higher value for most of the yield attributing characters. The similar result was reported by Swain *et al.,* 2014.

PEG directly affected the solute accumulation in cell; simultaneously there was a decline in physiological functions which in turn leads to reduced seedling weight. In developed cells, turgor pressure was drastically reduced, which leads to disrupted water flux from xylem to other cells. In new cell development, cell division and cell elongation was blocked. It resulted in reduced seedling weight and shoot length. It was in accordance with the findings of Chaturvedi *et al.,* 2012. Initial root length was considered

as good selection criteria for identifying mutants with altered drought response. Root length and number decreased with increased moisture stress. Among the mutants studied, 22 mutants had more number of roots than Nagina22 under drought stress. In case of root length, 201 mutants had more root length in stressed plant than control. It showed that elongated viable root system could help the plants to absorb water from deeper layers of soil. Hence, these mutants performed better under drought stress. For effective photosynthesis and translocation, longer roots will absorb more water and it helps the plants to survive under moisture stress. Inhibition of radicle or plumule was due to non-availability of water and imbalance in water potential gradient. Reduction in radical emergence showed impairment in seedling height (Sokoto and Muhammad, 2014). Based on IRRI Standard evaluation system, drought susceptibility was scored from 0 to 9 under drought stress. Visual score was increased with increased PEG concentration. At -10 bars, Nagina22 had a score of 7. Among the mutants studied, 32 mutants had lower visual score than Nagina22 at -6 bars. It showed that these mutants had osmotic balance under drought stress. Visual score of mutants ranged from 3 to 9 with an average of 6.5.
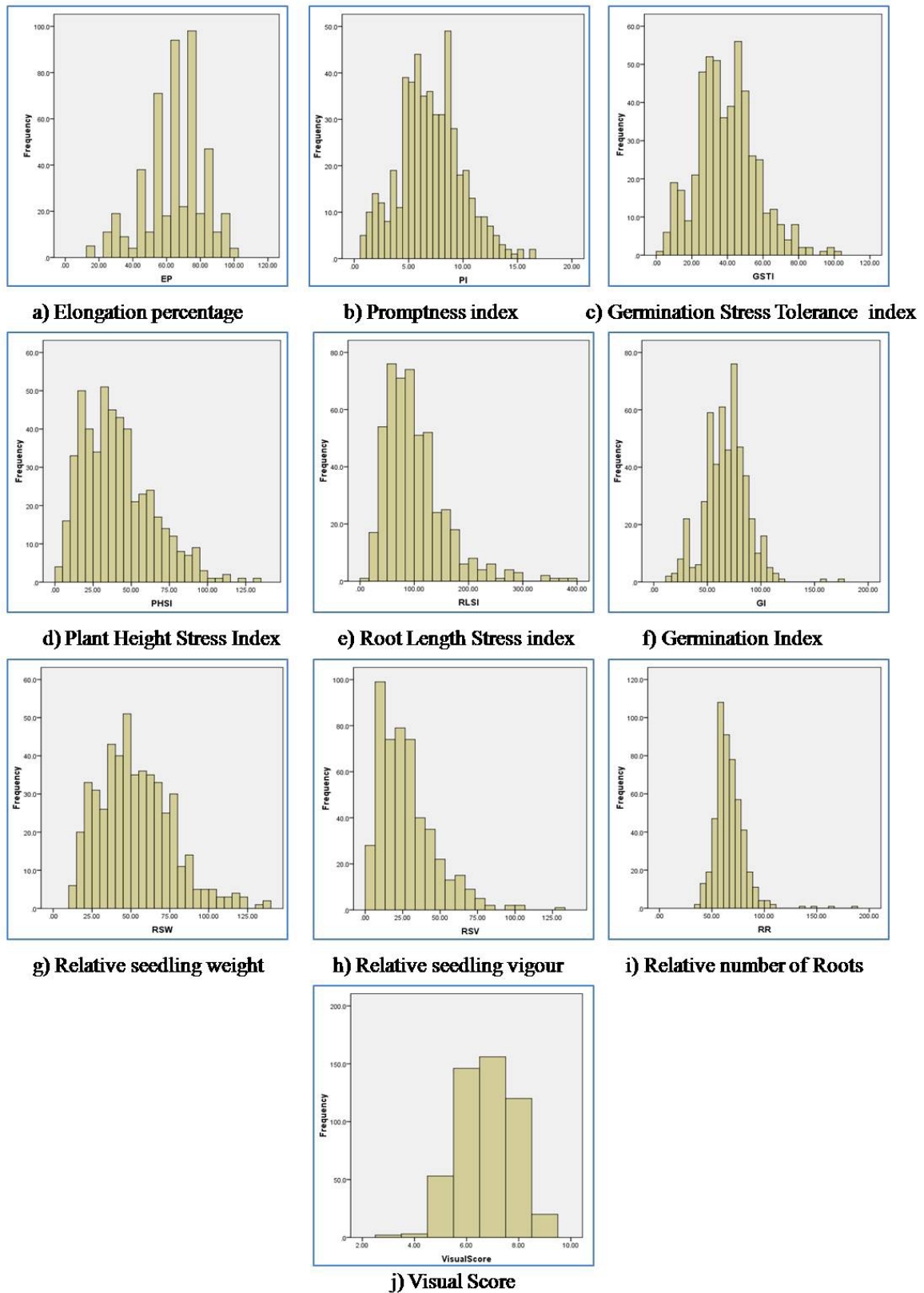
a) Elongation percentage
b) Promptness index
c) Germination Stress Tolerance index
d) Plant Height Stress Index
e) Root Length Stress index
f) Germination Index
g) Relative seedling weight
h) Relative seedling vigour
i) Relative number of Roots
j) Visual Score

Fig 2. Frequency distribution graph of Nagina22 mutants for different seedling traits and indices under drought stress
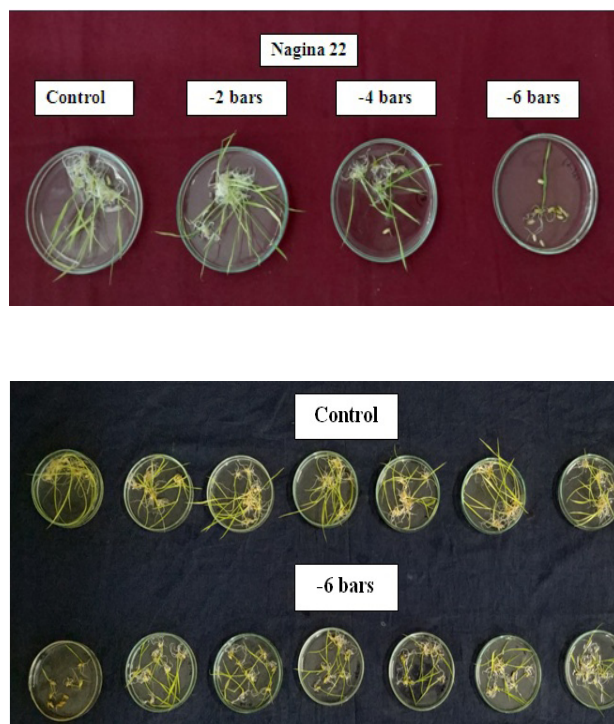
Fig 3.a) Standardization of PEG 6000 using Petri dish method and b) Screening of Nagina22 mutants in control and -6 bars

Seedling vigour is considered as selection criteria to identify the better mutants under drought stress. Relative seedling vigour calculated based on seedling length and emergence percentage under drought and control condition. It was also decreased with increased drought stress. Among the mutants, 109 mutants recorded highest RSV than Nagina22. Reduced seedling vigour was resulted from reduced seedling length and germination. Selection of mutants with altered drought response based on emergence percentage, promptness index, root length and seedling vigour will be highly effective. As a whole, two mutants N22-PDT-17 and N22-PDT-64 recorded highest value for all the characters studied except number of roots than Nagina22.

Correlation is the index of degree of relationship between two continuous variables. The correlation between ten germination and seedling related characters *viz.,* EP, PI, GSTI, PHSI, RLSI, GI, RSW, RR, Score and RSV were done using SPSS software (Table 4). RSV showed positive significant correlation with EP (0.430), PI (0.429), GSTI (0.464), PHSI (0.833), GI (0.498), RSW (0.346) and Score (0.072). Visual score showed positive correlation with PI (0.065), GSTI (0.065), PHSI (0.074) and RLSI (0.087). Number of roots showed positive correlation withGSTI (0.071) and RSW (0.131). Relative seedling weight showed positive correlation with EP (0.122), PI (0.131), GSTI (0.153), PHSI (0.301), RLSI

Table 4. Pearson's correlation coefficient among different seedling parameters and indices under drought stress in Nagina22 mutants

Traits	EP	PI	GSTI	PHSI	RLSI	GI	RSW	RR	Score	RSV
EP	1	0.830**	0.813**	-0.008	-0.009	0.925**	0.122**	0.007	0.008	0.430**
PI		1	0.963**	0.060	-0.019	0.766**	0.131**	0.041	0.065*	0.429**
GSTI			1	0.059	-0.010	0.825**	0.153**	0.071*	0.065*	0.464**
PHSI				1	-0.050	0.017	0.301**	0.002	0.074*	0.833**
RLSI					1	-0.012	0.073*	0.34	0.087**	-0.043
GI						1	0.155**	0.053	0.007	0.498**
RSW							1	0.131**	0.031	0.346**
RR								1	-0.031	0.016
Score									1	0.072*
RSV										1

(EP- Elongation percentage, PI- Promptness index, GSTI – Germination stress tolerance index, PHSI – Plant height stress index, RLSI – Root length stress index, GI – Germination index, RSW – Relative seedling weight, RR – Relative number of roots, Score – Visual score, RSV – Relative seedling vigour**, ** Significant at 1% probability, * Significant at 5% probability)

(0.073) and GI (0.155). Germination index showed positive correlation with EP (0.925), PI (0.766) and GSTI (0.825). Germination stress tolerance index showed positive correlation with EP (0.813) and PI (0.963). Promptness index showed positive correlation with EP (0.830). All other characters had no significant inter correlation with any of the traits studied. No negative correlation was recorded. The similar results were reported by Partheeban *et al.,* 2017.

Principal component analysis (PCA) is commonly used multivariate method. It is used to identify the minimum component which can explain maximum variation out of total variability (Anderson, 1972). First four principal components had eigen value more than 1 (Table 5). Those PCs had more variation and could be used for selection of drought tolerant mutants. PC1 had highest variation of about 39.5%, which is followed by PC2 (17.7%), PC3 (11.1%) and PC4 (10.6%). First two principal components revealed 57.2% variation for germination and growth

related indices. From the scree plot, it is observed that PC1 had maximum variation in comparison to other 9 PCs (Figure 4a). PC1 contributing traits were EP, PI, GSTI, RSW and RSV. PC3 contributing traits were RLSI, RSW and Score. The PC1 contributing traits were used as

selection criteria for identification of mutants with altered drought response. From the outlier plot, the mutants with high genetic distance were plotted beyond 4.29 (Fig. 4b). Similarly, drought tolerant genotypes were identified using multivariate analysis by Utharasu and Anandakumar,

Table 5. Eigen value, variability %, cumulative % and eigen factorsfor principal components under drought stress

S.No.	Parameters	PC1	PC2	PC3	PC4
1.	Eigen value	3.9513	1.7704	1.1147	1.0642
2.	Variability (%)	39.5	17.7	11.1	10.6
3.	Cumulative %	39.5	57.2	68.4	19.0
	Traits		**Factor loading**		
1.	EP	0.456	0.206	-0.027	0.011
2.	PI	0.458	0.170	0.002	0.036
3.	GSTI	0.467	0.158	0.024	0.013
4.	PHSI	0.132	-0.681	-0.148	0.050
5.	RLSI	-0.010	0.013	0.715	0.242
6.	GI	0.010	0.162	-0.009	-0.027
7.	RSW	0.458	-0.380	0.322	-0.231
8.	RR	0.141	-0.035	0.485	-0.646
9.	Score	0.034	-0.097	0.332	0.681
10.	RSV	0.338	-0.508	-0.127	0.039
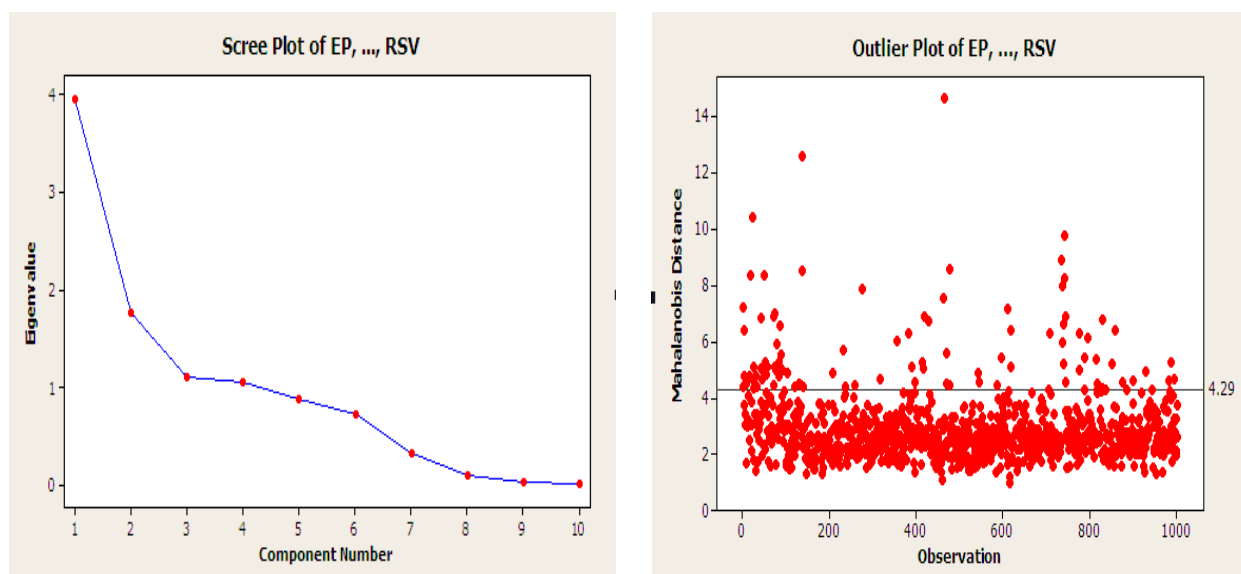
Fig 4a & b. Scree Plot and outlier plot showing mahalanobis distance between the mutants studied under PEG induced stress condition

2014. Hence, those mutants could be further evaluated and utilized in breeding programme.

In general, rice is sensitive to drought in all growth stages. Particularly, drought stress at germination and growth leads to reduced seedling establishment. As a result, reduced performance was observed in all other growth stages of rice. In vitro screening using polyethylene glycol is easy as well as more number of mutants can be screened at once. In this, two mutants N22-PDT-17 and

N22-PDT-64 recorded highest value for all the characters studied except number of roots than Nagina22. Hence, these mutants could be further evaluated under natural drought stress environment for further reliable and accurate results.

ACKNOWLEDGEMENT

Functional Genomics of Rice" funded by the Department of Biotechnology, Government of India.

REFERENCE

Anderson, T. W. 1972. An Introduction to Multivariate Analysis. Wiley Eastem Pvt. Ltd. New Delhi.

Ashraf, M., and Mehmood, S. 1990. Response of four Brassica species to drought stress.*Envir. Expt. Bot.*, **1**: 93-100. [Cross Ref]

Chaturvedi, G. S., Singh, A. and Bahadur R. 2012. Screening techniques for evaluating crop germplasm for drought tolerance. *Plant Archive*, **12**(1): 11–18.

Ellis, R.H. and Roberts, E. H. 1981.The quantification of ageing and survival in orthodox   seeds. *Seed science and Technology*, **9**:373-409

Gharoobi, B., M. Ghorbani, and Nezhad,M.G.. 2012. Effects of different levels of osmotic   potential on germination percentage and germination rate of barley, corn and canola.   *Iranian Journal of Plant Physiology*, **2**:413-417.

Govindaraj, M., Shanmugasundaram, P., Sumathi, P., and Muthiah, A.R. 2010. Simple, Rapid   and   Cost effective screening method for drought resistance breeding in pearl millet.   *Electronic Journal of Plant Breeding*. **1**(4):590-599.

International Rice Research Institute (IRRI). 1980. Standard Evaluation System for Rice, 2nd   e d i t i o n , International Rice Research Institute, Los Bafios, *The Philippines*, 44 pp.

Islam, M. M., Kayesh, E., Zaman, E., Urmi, T. A. and Haque,M. M. 2018. Evaluation of rice (*Oryza   sativa*   L.) genotypes for drought tolerance at germination and early seedling stage.   *The   Agriculturists*. **16**(1): 44-54. [Cross Ref]

Pandey V. andShukla A. 2015. Acclimation and tolerance strategies of rice under drought stress. *Rice Science*, **22**(4): 147–161. [Cross Ref]

Partheeban, C., Chandrasekhar,C.N., Jeyakumar,P., Ravikesavan,R. and Gnanam, R. 2017. Effect of PEG Induced Drought Stress on Seed Germination and Seedling Characters of Maize (*Zea mays* L.). Genotypes. Int. J. Curr. Microbiol. *App. Sci.* **6**(5): 1095-1104. [Cross Ref]

Petrovic, G., Jovicic, D., Nikolic, Z., Tamindzic, G., Ignjatov, M., Milosevic, D. and Milosevic, B. 2016. Comparative study of drought and salt stress effects on germination and seedling growth of pea. Genetika, **48**(1): 373-381. [Cross Ref]

Sokoto, M.B. and Muhammad, A. 2014. Response of rice varieties to water stress in Sokoto,   S u d a n Savannah, Nigeria. *Journal of Bioscience Med*, **2**: 68–74. [Cross Ref]

Swain, P., Anumalla, M., Prusty, S., Marndi, B.C. and Rao,G.J.N. 2014. Characterization of s o m e native landrace rice accessions for drought tolerance at seedling stage. *Australian Journal of Crop Science*, **8**(3): 324-331.

Taiz, L. and Zeiger, E. (2006). Plant physiology 4ᵗʰedn. Sunderland, MA: *Sinauer Associates* :7- 64.

Utharasu, S., andAnandakumar, C. R. 2014. In-vitro screening and field validation of rice (Oryza   sativa   L.) genotypes for drought tolerance. *Electronic Journal of Plant Breeding*, **5**(1): 1-10.