The Andhra Agric. J 66 (4): 623-627, 2019

Screening of $F_{2:3}$ Mapping Population of Swarna Sub1 / AC39416A for Anaerobic Germination in Rice (*Oryza Sativa* L.)

B Sai Mohan, N Chamundeswari, T Haritha and N Veronica
Department of Genetics and Plant Breeding, Agricultural College, Bapatla, A. P.

ABSTRACT

Phenotypic screening for anaerobic germination (AG) was conducted at RARS, Maruteru, West Godavari district of Andhra Pradesh, using 188 $F_{2:3}$ biparental cross mapping population of Swarna sub1 / AC39416A along with parentsas per standard protocol in Complete Randomized Design (CRD) with two replications. Anaerobic stress was created by submergence of trays with 15 cm depth of water in concrete tank for 14 and 21 days in two separate experiments. The level of tolerance to submergence during germination *i.e* AG percent in the population was recorded. After one week of de-submergence the observations *viz.,* plant survival percent, seedling shoot length (cm) and seedling root length (cm) were recorded. Analysis of variance revealed significant variation for all the characters studied. Among 188 $F_{2:3}$ population screened, only 47 lines (for two weeks of submergence) and 17 lines (for three weeks of submergence) had shown more than 70% of AG. The mean AG (%) ranged between 0% and 95% in the population.

Key words: *Anaerobic germination, Biparental cross population, Submergence.*

The slogan called 'Rice is life' is the most appropriate for India, where this crop provides staple food as well as livelihood for millions of people. Rice contributes 43% and 46% of total food grain and cereal production of the country respectively. It is estimated that by the year 2030, the rice demand for internal consumption will be 121.2 million tonnes (Ram *et al*., 2018).

Production and productivity of Rice is majorly effected by abiotic stresses particularly floods under coastal irrigated ecosystem because of unexpected heavy rainfall, which is a major climate change challenge that often place a major limitation on rice cultivation (Reddy *et al*., 2015). Flooding during seed germination may be a consequence of unevenly levelled ûelds or early and unforeseen rains, or even, when rice ûelds are purposely ûooded after sowing to combat weeds. Rice is the only major cereal that exhibits some degree of tolerance to anaerobic conditions during germination (AG), which is limited to coleoptile emergence and partial growth, but not sufficient to overcome the stress (Miro *et al*., 2017). Among all abiotic stress, tolerance to flooding during the process of seed germination *i.e* anaerobic germination (AG) tolerance is the rarest phenomenon (Zhang *et al*., 2018). Tolerance of rice crop to flooding stress through enhanced germination and early growth of the seedlings is a prerequisite for successful cultivation in regions where floods is a recurrent problem. Although rice could tolerate flooding, its germination is limited to coleoptiles elongation as in susceptible genotypes, root and primary leaf fail to develop normally (Kumar *et al*., 2018). Hence, enhanced flooding tolerance during germination and early seedling growth could therefore help to improve crop stand establishment and promote widespread adoption of direct seeded cultivation in both rainfed and irrigated ecosystems (Chamara *et al*., 2018). Keeping in view the importance of AG, the present study was taken up to screen the 188 $F_{2:3}$ biparental cross population of the contrasting parents Swarna sub1 and AC39416A for AG, where Swarna sub1 (Recepient parent) is susceptible and AC39416A (Donor parent) is tolerant to submergence stress during germination.

MATERIAL AND METHODS

The biparental cross between Swarna sub1 and AC39416A was made during *Rabi* 2016-17 and F_1 was raised during *Kharif* 2017. The F_2 was evaluated during *Rabi* 2017-18 and 188 single plants were selected randomly for screening of AG. Phenotypic screening for 188 $F_{2:3}$ population of Swarna sub1 / AC39416A along with parents was conducted during *Kharif* 2018 at RARS, Maruteru, West Godavari district of Andhra Pradesh, in complete randomized block design with two replications in concrete tank as per Septiningsih*et al*. (2013).

From each line about 25 healthy (*i.e* completely filled) seeds were soaked for a period of about 24 hours and kept for incubation in dark or closed chamber again for 24 hours so that seeds will start germinating. Then pre-germinated seeds were sown in pro-trays which are filled with well puddled soil in such a way that the sprouted portion facing upwards or top end. Finally the pro-trays were arranged randomly inside the concrete tank and submergence (stress) is imposed by filling water up to 15 cm above the trays. Constant depth of water is maintained throughout the submergence treatment *i.e*for two weeks and three weeks in separate experiments. After submergence treatment for two and three weeks separately, the pro-trays were kept outside of the concrete tank and allowed for one week of de-submergence treatment during which trays were watered daily.

Data was recorded from survived seedlings of $F_{2:3}$ population of Swarna sub1 / AC39416A along with two parents as detailed below.

Anaerobic germination (%) after two weeks of submergence

Germinated Seedlings were counted at 14 DAS in submergence tank and percent anaerobic germination was determined (Manigbas*et al*., 2008). The genotypes which recorded more than 75% of germination under anaerobic conditions are considered as tolerant to anaerobic germination (Reddy *et al*., 2015).

Plant survival (%) one week after de-submergence

The seedlings survived after one week of de-submergence period following two weeks and three weeks of submergence were noted and number of seedling survived was represented as plant survival percent.

Seedling shoot length (cm) one week after de-submergence

Shoot length from five randomly selected seedlings in each of the experiment after one week of de-submergence following two weeks and three weeks of submergence was measured and is expressed in centimetres.

Seedling root length (cm) one week after de-submergence

The root length was recorded in five randomly selected seedlings in each of the experiment after one week of de-submergence period following two weeks and three weeks of submergence and expressed in centimetres.

STATISTICAL ANALYSIS

The mean data collected in respect of above observations was subjected to the following statistical techniques as per the standard statistical procedure given Gomez and Gomez. (1984). given in the (Table 5). The treatment mean sum of squares was tested against error mean sum of square to find out the significance.

RESULTS AND DISCUSSION

The analysis of variance for all the four characters *viz*., anaerobic germination %, plant survival % one week after de-submergence, seedling shoot length (cm) and seedling root length (cm) for two and three weeks of submergence period revealed significant differences which indicates that there is a significant variation for parameters studied among the $F_{2:3}$ population.

Anaerobic germination (%)

The number of seedlings survived after submergence treatment were counted and expressed as anaerobic germination percent. The mean anaerobic germination per cent recorded after two weeks of submergence among the population of 188 lines ranges from 0 to 95% with overall mean of 47.51%. Whereas mean AG per cent of the contrasting parents Swarna sub1 and AC39416A was 40% and 85% respectively. In case of three weeks of submergence treatment, the mean anaerobic germination per cent recorded was ranged from 0 to 95%, with overall mean of 37.66% and the mean AG per cent of the two contrasting parents Swarna sub1 and AC39416A was 27% and 75.6%, respectively, (Table 1) indicating that there is wider variation in the mean performance of population for anaerobic germination tolerance. Similar trend of results were reported by Barik *et al*. (2019). Greater variability in germplasm lines screened for anaerobic germination was described by Umarani*et al*. (2018).

Plant survival (%) after one week of de-submergence

Plant survival rate was calculated by counting the seedlings survived aften one week of de-submergence following submergence treatment. The average survival rates after two weeks of submergence period of the parents , Swarna sub1 and AC39416A was 35% and 80% respectively. While survival percent ranged from 0 to 95% in their population with overall mean of 36.74%. Whereas, after three weeks of submergence the average survival rates of the two contrasting parents Swarna sub1 and AC39416A was 17.6% and 72% respectively. The survival rates ranged from 0 to 90%, with overall mean of 15.55% in the population screened for tolerance of submergence during germination stage and inferring that variation was observed clearly in the population between the two extreme phenotypes (Table 2). Doley*et al*., (2018) noticed that survival per cent was correlated positively with coleoptiles elongation which helps in obtaining

Table 1. Frequency distribution of anaerobic germination % in $F_{2:3}$ population

S.No	Anaerobic Germination (%)	No of $F_{2:3}$ lines	
		2 Weeks of submergence	3 Weeks of submergence
1	0-10	27	32
2	11-20	26	24
3	21-30	13	34
4	31-40	16	19
5	41-50	13	21
6	51-60	23	27
7	61-70	23	17
8	71-80	18	8
9	81-90	22	4
10	91-100	7	2

Table 2. Frequency distribution of plant survival % after one week of de-submergence in $F_{2:3}$ population

S.No	Plant Survival (%)	No of $F_{2:3}$ lines	
		2 Weeks of submergence	3 Weeks of submergence
1	0-10	56	97
2	11-20	15	37
3	21-30	14	27
4	31-40	19	12
5	41-50	19	10
6	51-60	21	3
7	61-70	22	1
8	71-80	14	0
9	81-90	6	1
10	91-100	2	0

Table 3. Frequency distribution of seedling root length (cm) in F_2 population

S.No	Root Length (cm)	No of $F_{2:3}$ lines	
		2 Weeks of submergence	3 Weeks of submergence
1	0	35	52
2	1-3	5	48
3	3-6	40	74
4	6-9	103	14
5	>9	5	0

Table 4. Frequency distribution of seedling shoot length (cm) in F_2 population

S.No	Shoot Length (cm)	No of $F_{2:3}$ lines	
		2 Weeks of submergence	3 Weeks of submergence
1	0	35	52
2	1-6	0	44
3	6-12	14	72
4	12-18	94	17
5	18-24	55	3

Table 5. Phenotypic screening for anaerobic germination

	2 Weeks of Submergence				3 Weeks of Submergence			
	AG(%)	PS(%)	RL(cm)	SL(cm)	AG(%)	PS(%)	RL(cm)	SL(cm)
Minimum	0	0	0	0	0	0	0	0
Maximum	95	95	9.96	24.5	95	90	8.9	21.9
Mean	47.51	36.74	5.48	13.34	37.6	15.54	2.73	6.01
CV%	6.11	8.8	8.3	5.09	4.29	5.65	8.3	5.54
CD	4.78	5.9	0.9	1.33	2.96	2.12	0.44	0.65
SE(m)	1.71	2.11	0.32	0.48	1.06	0.76	0.16	0.23

C.V (%) = Coefficient of Variation
C.D. = Critical Difference
SE (m) = Standard Error of Mean
AG (%) = Anaerobic Germination
PS (%) = Plant Survival
RL(cm) = Seedling Root Length
SL (cm) = Seedling Shoot Length

oxygen from surroundings. Similar pattern of variation in survival per cent of population was described by Septiningsih*et al*., (2013) in the $F_{2:3}$ population of IR 64 and Ma-Zhan Red and Baltazar *et al*. (2014) in $F_{2:3}$ population of IR 64 and Nanhi during screening for tolerance to anaerobic conditions during germination. Whereas results reported by Angaji (2008) shows less variation in the BC_2F_2 population of IR64/ KHAIYAN screened for tolerance during germination stage submergence.

Seedling shoot length (cm) after one week of de-submergence

Rice coleoptiles and shoots are one of the few plant organs that could be able to grow under absence or low oxygen conditions and vary greatly for rates of elongation and alcoholic fermentation. Rapid elongation of coleoptiles and shoots could facilitate, to get contact with air under anaerobic soils and further help to supply oxygen for growth of the embryo. Hence, shoot length of seedlings including the coleoptiles was used as marker to determine anaerobic germination tolerance as it is difficult to detect amount of alcohol under anoxia conditions (Ling *et al*., 2004).

Seedling shoot length of randomly selected seedlings survived after one week of de-submergence following submergence treatment is expressed in centimetres. The mean shoot length of the population was ranged from 0 to 24.05 cm with overall mean of 13.34 cm and the mean seedling shoot length of the two parents, Swarna sub1 and AC39416A was 10.33 cm and 16.6 cm, respectively in two weeks of submergence treatment. While in case of three weeks of submergence treatment, the mean shoot length of the population ranged between 0 and 21.90 cm with an overall mean of 6.01 cm, indicating greater variability in population. The mean seedling shoot length of the parents Swarna sub1 and AC39416A, were 9.17 cm and 14.3 cm, respectively (Table 3). Seedlings show more elongation due to influence of anaerobic responsive enzymes particularly more in case of cereals (rice) to expose seedlings to oxygen. Barik *etal*. (2019) noticed lesser variation in the mean shoot length of survived seedlings screened landraces for anaerobic germination tolerance.

Seedling root length (cm) after one week of de-submergence

The mean seedling root length recorded after one week of de-submergence followed by two weeks of submergence treatment among the population ranged from 0 to 9.96 cm with an overall mean of 5.47 cm, whereas parents Swarna sub1 and AC39416A recorded 6.5 cm and 7.9 cm of mean seedling root length respectively. The mean seedling root length recorded after one week of de-submergence period following three weeks of submergence treatment was ranged from 0 to 8.92 cm, while the parents, Swarna sub1 and AC39416A recorded 4.9 cm and 6.1 cm of mean seedling root length respectively (Table 4). Increased seedling root and shoot growth was observed by Ismail *et al*. (2011) while screening for anaerobic germination. Results reported by Barik *etal*. (2019) were also in agreement with the results of the present investigation.

CONCLUSION

Among the material screened, 47 lines (for 2 weeks of submergence) and 17 lines (for 3 weeks of submergence) had shown more than 70% of AG. 12 lines for two weeks treatment and five lines for three weeks treatment have shown AG on par with the donor parent AC39416A. The material screened in the present experiment will be useful for mapping of QTLs for AG in future.

LITERATURE CITED

Angaji S A 2008 Mapping qtls for submergence tolerance during germination in rice. *African Journal of Biotechnology.* 7 (15): 2551-2558.

Baltazar M D, Ignacio J C I, Thomson M J, Ismail A M and Septiningsih E M 2014 QTL mapping for tolerance of anaerobic germination from IR64 and the aus landrace Nanhi using SNP genotyping. *Euphytica*. 197: 251-260.

Barik J, Kumar V, Lenka S K and Panda D 2019 Genetic potentiality of lowland indigenous indica rice (Oryzasativa L.) landraces to anaerobic germination potential. *Indian Journal of Plant Physiology*. pp: 1-13.

Chamara B S, Marambe B, Kumar V, Ismail A M, Septiningsih E M and Chauhan B S 2018 Optimizing sowing and flooding depth for anaerobic germination-tolerant genotypes to enhance crop establishment, early growth, and weed management in dry-seeded rice (*Oryza sativa* L.). *Frontiers in plant science*. *9*: 1-15.

Doley D, Barua M, Sarma D and Barua P K 2018 Screening and enhancement of anaerobic germination of rice genotypes by pre-sowing seed treatments. *Current Science*. 115 (6): 1185-1190.

Gomez K A and Gomez A A 1984 *Statistical procedures in Agricultural Research.* New York Chichester Wiley. 2nd *edition*. 680.

Ismail A M, Ella E S and Dionisio-Sese M L 2011 Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. *AoB Plants*. 2011: 1-11.

Kumar D, Das P K and Sarmah B K 2018 Reference gene validation for normalization of RT-q PCR assay associated with germination and survival of rice under hypoxic condition. *Journal of Applied Genetics*. 59: 419–430.

Ling J, Ming-yu H W, Ming C and Jian-min W 2004 Quantitative trait loci and epistatic analysis of seed anoxia germinability in rice (*Oryza sativa* L.). *Rice Science*. 11 (5-6): 238-244.

Manigbas N L, Solis R O, Barroga W V, Noriel A J, Arocena E C, Padolina T F and Cruz R T 2008 *Philippine Journal of Crop Science*. 33 (3): 34-44.

Miro B, Longkumer T, Entila F D, Kohli A and Ismail A M 2017 Rice seed germination underwater: Morpho-physiological responses and the bases of differential expression of alcoholic fermentation enzymes. *Frontiers in plant science*. 8: 1-17.

Ram B J, Babu G S, Singh O N, Lavanya G R and Kumar K M 2018 Molecular characterization of rice genotypes using microsatellite markers. *Journal of Pharmacognosy and Phytochemistry*. 7 (5): 3065-3069.

Reddy V A, Girija Rani M, Satyanarayana P V, Suryanarayana Y, Chamundeswari N, Ravikumar B N V S R, Ramana R P V and Vishnuvardhan K M 2015 Physiological and molecular response of rice genotypes for different types of flooding. *Current Biotica*. 8 (4): 345-350.

Septiningsih E M, Ignacio J C I, Sendon P M D, Sanchez D L, Ismail A M and Mackill D J 2013 *QTL* mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. *Theoretical and Applied Genetics.* 126: 1357–1366.

Umarani E, Hemalatha V, Bhadana V P, Senguttuvel P, Subbarao L V, Neeraja C N, Ravi P, Yugandhar and Kota S 2018 Screening of Rice (*Oryza sativa* L.) Genotypes for Anaerobic Germination. *International Journal of Pure and Applied Bioscience.* 6 (5): 1318-1325.

Zhang J and Rahman A R B 2018 Preferential geographic distribution pattern of abiotic stress tolerant rice. *Rice*. 11 (1): 1-16.

Received on 27.02.2019 and revised on 06.02.2020