International Journal in Physical and Applied Sciences
Volume 5 Issue 08, August 2018 ISSN: 2394-5710 Impact Factor: 4.657
Journal Homepage: http://ijmr.net.in, Email: irjmss@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

SCREENING OF RICE BREEDING LINES FOR FLOODED WATER CONDITION

Edward Momoh Munu[1,2],Aiah Steven Ngaujah[2], Sayo Sesay[2] and AlieuMohamed Bah[1] *

[1]Department of Crop science, School of Agriculture, N'jala University, N'jala Campus, Sierra Leone

[2]Sierra Leone Agricultural Research Institute (SLARI)-Rokupr Agricultural Research Centre (RARC), PMB 1313, Freetown Sierra Leone

Abstract

Screening of F3-F4 rice breeding lines for adaptation to flooded water condition is helpful in increasing rice production efficiency in flood prone agro-ecologies like Riverain grasslands, Bolilands Mangrove swamps and Inland valley swamps; that possess enormous potential for enhancing food production to help meet the ever-increasing demands for rice. These ecologies are predominantly characterized by fertile soils and fresh water resources that are critical in increasing rice yields; yet farmers suffer huge losses due to low yields. 60 breeding lines developed through crosses in 2014 at RARC using spikelet emasculation method were screened in addition to 4 submergence tolerant varieties used as Checks (2 improved and 2 traditional varieties) for adaptation to flooded or standing water condition. Transparent 35cm diameter buckets filled with equal quantity of properly dried soil collected from Inland valley Swamp. 15 seeds for each breeding line including the check varieties were sown and subjecting them under anaerobic germination conditions using equal amount of tap water, thus submerging the seeds at a water depth of 20cm from the soil surface. The above setup was repeated at 10cm water depth in (second set). ASimilar setup was subjected under aerobic germination conditions. The finding shows that, Line C6P11, C6P10, C8P17 and C8P12 had the highest anaerobic germination and regeneration percentages at both levels (20cm and 10cm water depths) showed more tolerance to flooded or standing water conditions. They also exhibited fast recovery ability in terms of plant height and leaf chlorophyll concentration on leaves when compared to breeding lines subjected under aerobic germination condition. Breeding lines with good adaptability to flooded water conditions germinated under water and had the slowest or limited shoot elongation and also exhibited fast recovery ability after desubmergence that could be used as indices for evaluating adaptable breeding lines. This experiment should be repeated in replicated trials in laboratory or screen house and field to confirm conclusions made.

Key words: Breeding lines, Submergence, Aerobic, Anaerobic, Regeneration

1.0: INTRODUCTION

Rice is the most important food crop in the world. It is consumed by nearly 3 billion people almost daily (Huke and Huke 1997; Ito et al., 1999). The crop sub sector in Sierra Leone is dominated with rice which is the country's staple food. Rice contributes about 75 percent of agricultural Gross domestic Product (GDP). Annual per capita consumption of rice (104 kg) in Sierra Leone is among the highest in sub Saharan Africa. There are five rice growing ecologies in Sierra Leone which includes Upland, Inland Valley Swamps, Mangrove Swamps, Bolilands, and the Riverain Grasslands. Among the above listed ecologies, Inland Valley Swamps, Mangrove Swamps, Bolilands, and the Riverain Grasslands generally categorized as lowland ecologies. Excess water is common in most lowland rice production areas in Sierra Leone with particular reference to Riverain grasslands and Bolilands. These ecologies have the potential for increase in rice cultivation due to their relatively high native fertility, low incidences of pests and diseases, as well as their suitability for tractorisation.

A submerged plant is defined as "a plant standing in water with at least part of the terminal above the water or completely covered with water" (Catling 1992). However, complete submergence due to frequent flooding, accompanied by heavy down pour of rains, can adversely affect plant growth and yield. Floods seem to be worsening in recent years, due to effects commonly attributed to climate change, such as sea-level rise, the uneven distribution of rains and periodic changes in frequencies and intensities of floods caused by extreme weather events (Coumou and Rahmstorf, 2012).

Climate change which has become a worldwide threatening phenomenon is contributing actively to increased flooding, drought, soil degradation and reduction in fertility (FAO, 2011).Rice farmers in Sierra Leone working at Riverain grasslands and Bolilands, reported vast stretch of arable land, which suffers from stagnant flooding annually and repeatedly low yields of about 500Kgha^{-1} are being obtained from the currently grown cultivars. To exacerbate this situation, majority of the farmers especially those doing work in these ecologies establish their crop by direct seeding, which makes the crop more vulnerable to prolonged flood conditions resulting to poor crop stand establishment and in some cases total crop loss becomes eminent.

Floods are therefore considered major challenges for rice production in rainfed lowlands in Sierra Leone, where the majority of the rice farmers live and depend on rice and rice-based farming as their major source of food, income and livelihood. Also, limited research has been done on deep water rice or flooded condition. Tolerance of anaerobic conditions at these early stages is a prerequisite for effective direct-seeded rice in rainfed and flood-affected areas in Sierra Leone. More sustainable and permanent solutions are needed to overcome this problem. One of the most promising solutions is to develop high-yielding varieties that are submergence tolerant and are more likely to be rapidly adopted by the farmers in the target regions (Xu and Mackill, 1996). Thus the need to search for appropriate varieties that combine high yields with submergence tolerant and elongation ability is vital. Despite being a semi-aquatic species, rice is sensitive to various types of flooding stress and this sensitivity varies with the genotype, stage of development, duration and depth of flooding, and floodwater conditions (Daset al. 2009; Mackillet al. 2012). Even shallow flooding of the soil caused by heavy rainfall soon after seeding can reduce germination and result in poor crop establishment, since rice is sensitive to flooding during germination (Yamauchi et al. 1993). Slow seed germination and delayed seedling establishment will become a major problem for rice production in flood-prone lowland areas as sowing method shifts from transplanting to direct seeding. Enhancement in the genotypic tolerance to anaerobic conditions during germination is much more inexpensive for poor farmers in developing countries and is more feasible for adoption on a larger scale than other management practices. Unfortunately, very limited success has been achieved from previous efforts to improve the tolerance of genotypes for anaerobic conditions during germination (Jiang et al., 2006).

Plant responses to flooding during germination was observed by Ismail et al,(2009). They monitored coleoptile elongation in some different rice varieties.When flooding occurs just after direct seeding, tolerant rice genotypes germinate faster and their coleoptile grow at a relatively faster rate to emerge from flooded soils.Plant height is also an important trait of submergence-tolerant rice genotypes that gives an indication of shoot elongation rate or fast recovery rate (Takai et al.,2010).These genotypes are also capable of forming roots and leaves in shallow water depths (Angaji et al.,2010).Rice genotypes adapted to this type of flooding usually stay dormant or ‘quiescent’ when flooded to conserve their energy reserves and maintain their chlorophyll and underwater photosynthesis (Winkelet al. 2013.Chlorophyll concentration is an important trait in submergence-tolerant genotypes. A similar result was reported by Ella and Ismail (2006), Fukao et al. (2006)which shows that the ability of the tolerant genotypes to maintain higher chlorophyll during and after submergence is critical for survival since it ensures underwater photosynthesis and faster recovery after the water recedes. Breeding for better germination, greater seedling vigour and higher tolerance of waterlogging in rice has been attempted before, but with limited success because genotypes with sufficient tolerance were not available (Ling et al. 2004). It was also obserevd that, responses of plant in hypoxic conditions are influenced by certain hormones (Fukao et al. 2006).Unfortunately, very limited success has been achieved from previous efforts to improve the tolerance of genotypes for anaerobic conditions during germination (Jiang et al., 2006). For instance, Angajiet al. (2009) reported that tolerance to flooding during germination seems relatively rare in riceafter screening over 8000 gene bank accessions, elite breeding lines and genotypes, they identified few genotypes with greater ability to germinate under flooding condition.In submergence-prone areas (10-12days under water) with no soil problems, yield can be doubled with the use of submergence tolerant improved varieties (IRRI, 2006).This is in line with the objective of this study which is to identify rice varieties with high yield potential and adaptable to lowland flood-prone rice-growing environments in Sierra Leone.

2.0: MATERIALS AND METHODS

2.1: seeds collection

Seeds were obtained from 64 breeding lines of which 60 were developed through conventional crosses using spikelets emasculation carried out at the Rokupr Agricultural Research Centre (RARC) and whose parentage are tolerant to submergence stress. The remaining 4 breeding lines which were used as checks are submergence tolerant varieties (2 RARC improved varieties and 2 Farmers' varieties).

Table 1: Variety list of the trial

LINE NO.	LINE CODE	PARENTAGE	
		FEMALE	MALE
1	C1P1	IR64 + SUB 1	ROK16
2	CIP2	IR64 + SUB 1	ROK16
3	C1P3	IR64 + SUB 1	ROK16
4	C2P1	ROK 16	IR64+SUB 1
5	C2P2	ROK 16	IR64+SUB 1
6	C2P3	ROK 16	IR64+SUB 1
7	C5P1	ROK 16	TDK + SUB 1
8	C5P2	ROK 16	TDK + SUB 1
9	C5P3	ROK 16	TDK + SUB 1
10	C5P4	ROK 16	TDK + SUB 1
11	C5P5	ROK 16	TDK + SUB 1
12	C5P6	ROK 16	TDK + SUB 1
13	C5P7	ROK 16	TDK + SUB 1
14	C5P8	ROK 16	TDK + SUB 1
15	C5P9	ROK 16	TDK + SUB 1
16	C5P10	ROK 16	TDK + SUB 1
17	C5P11	ROK 16	TDK + SUB 1
18	C5P13	ROK16	TDK + SUB 1
19	C6P1	Samba Mashuri 2 + SUB 1	ROK 24
20	C6P2	Samba Mashuri 2 + SUB 1	ROK 24
21	C6P3	Samba Mashuri 2 + SUB 1	ROK 24
22	C6P4	Samba Mashuri 2 + SUB 1	ROK 24
23	C6P6	Samba Mashuri 2 + SUB 1	ROK 24
24	C6P8	Samba Mashuri 2 + SUB 1	ROK 24
25	C6P9	Samba Mashuri 2 + SUB 1	ROK 24
26	C6P10	Samba Mashuri 2 + SUB 1	ROK 24

27	C6P11	Samba Mashuri 2 + SUB 1	ROK 24
28	C6P12	Samba Mashuri 2 + SUB 1	ROK 24
29	C7P1	ROK 24	Samba Mashuri 2 + SUB 1
30	C7P2	ROK 24	Samba Mashuri 2 + SUB 1
31	C7P3	ROK 24	Samba Mashuri 2 + SUB 1
32	C7P4	ROK 24	Samba Mashuri 2 + SUB 1
33	C7P5	ROK 24	Samba Mashuri 2 + SUB 1
34	C7P6	ROK 24	Samba Mashuri 2 + SUB 1
35	C7P7	ROK 24	Samba Mashuri 2 + SUB 1
36	C7P8	ROK 24	Samba Mashuri 2 + SUB 1
37	C7P9	ROK 24	Samba Mashuri 2 + SUB 1
38	C7P10	ROK 24	Samba Mashuri 2 + SUB 1
39	C8P1	ROK 24	Swarna + SUB 1
40	C8P2	ROK 24	Swarna + SUB 1
41	C8P3	ROK 24	Swarna + SUB 1
42	C8P4	ROK 24	Swarna + SUB 1
43	C8P5	ROK 24	Swarna + SUB 1
44	C8P6	ROK 24	Swarna + SUB 1
45	C8P7	ROK 24	Swarna + SUB 1
46	C8P8	ROK 24	Swarna + SUB 1
47	C8P9	ROK 24	Swarna + SUB 1
48	C8P10	ROK 24	Swarna + SUB 1
49	C8P11	ROK 24	Swarna + SUB 1
50	C8P12	ROK 24	Swarna + SUB 1
51	C8P13	ROK 24	Swarna + SUB 1
52	C8P14	ROK 24	Swarna + SUB 1
53	C8P15	ROK 24	Swarna + SUB 1
54	C8P16	ROK 24	Swarna + SUB 1
55	C8P17	ROK 24	Swarna + SUB 1
56	C8P18	ROK 24	Swarna + SUB 1
57	C8P19	ROK 24	Swarna + SUB 1
58	C8P20	ROK 24	Swarna + SUB 1
59	C9P1	ROK 24	TDK + SUB 1
60	C9P2	ROK 24	TDK + SUB 1
61	SambaMashuri 2 + Sub 1	SOURCE:	AfricaRice (Introduction)
62	Swarna + Sub 1	SOURCE:	AfricaRice (Introduction)
63	Korgbati	SOURCE:	Tormabum (Local collection)
64	Galawah	SOURCE:	Tormabum (Local collection)

2.2: soil collection and preparation of planting buckets

Transparent large sized Rubber buckets measuring 32 cm deep and a diameter of 30 cm were filled with equal quantity of dry Inland Valley swamp (IVS) soil which was thoroughly sun dried for 3 days and crushed to break the large clods of soil. A litre of water was measured to moisten the soil in each bucket in readiness for planting or sowing.

2.3: Treatment:

LEVEL 1: 15 seeds were counted from eachbreeding line and seeded in each compartment in the large-sized buckets. The seeds in the large-sized buckets were immediately submerged after seeding by adding 7.5 litres of tap water to each bucket thereby submerging the seeds to a depth of 20 cm from the soil surface. The position of each rubber bucket was changed on a daily basis to make sure that the effect of light is normalized. Equal number of seeds from each breeding line was also seeded in the small rubber buckets and allowed to germinate and grow under optimal conditions

LEVEL 2: Fifteen seeds were also obtained from each breeding line and seeded in each compartment in the big rubber buckets. After seeding, they were immediately submerged to a depth of 10cm from the soil surface using 3.75 litres of tap water. The position of each rubber bucket was also changed on a daily basis.

2.4: Data collection

Data collection started immediately after 14 days of submergence and 10 days after desubmergence. Number of germinated seeds /germination percentage was calculated by counting the number of germinated seeds and expresses it as a percentage of the total number of seeds sown at14 days of submergence.. Number of survived plants after 10 days of desubmergence was counted and expressed as a percentage of the total number of seeds sown. Height of plants were measured using a ruler graduated in centimeters at 10 days after desubmergence. Leaf Chlorophyll concentration percentage was obtained on the topmost leaves using a portable SPAD 502 meter.

2.5: Statistical Analysis

Basic statistics were used to analyze the data. All data were analyzed using Excel (2010) software package.

3.0: RESULTS

Table 2: Aerobic and Anaerobic germination percentages of F3-F4 breeding lines at 20cm and 10cm water depths at 14 days

Line code	Aerobic Set Germination %	Anaerobic germination % (20cm water depth)	Anaerobic germination % (10cm water depth)
C1P1	46.7	0	0
C1P2	53.3	0	0
C1P3	0	0	0
C2P1	13.3	6.7	0
C2P2	60	0	0
C2P3	60	0	0
C5P1	26.7	0	0
C5P2	73.3	6.7	6.7
C5P3	86.7	13.3	20

C5P4	46.7	0	0
C5P5	80	0	0
C5P6	40	0	0
C5P7	40	0	0
C5P8	86.7	0	0
C5P9	93.3	0	6.7
C5P10	40	0	0
C5P11	73.3	0	0
C5P13	80	0	0
C6P1	80	13.3	33.3
C6P2	13.3	0	0
C6P3	86.7	0	0
C6P4	80	0	0
C6P6	26.7	0	0
C6P8	33.3	0	0
C6P9	93.3	0	6.7
C6P10	93.3	33.3	46.7
C6P11	100	73.3	66.7
C6P12	80	26.7	40
C7P1	6.7	6.7	0
C7P2	33.3	0	0
C7P3	46.7	13.3	0
C7P4	80	0	6.7
C7P5	13.3	6.7	13.3
C7P6	60	33.3	40
C7P7	80	0	0
C7P8	86.7	0	0
C7P9	20	13.3	26.7
C7P10	53.3	0	0
C8P1	86.7	0	33.3
C8P2	86.7	0	13.3
C8P3	46.7	0	0
C8P4	66.7	6.7	0
C8P5	6.7	0	0
C8P6	73.3	0	6.7

C8P7	20	6.7	0
C8P8	73.3	6.7	6.7
C8P9	86.7	26.7	13.3
C8P10	53.3	6.7	26.7
C8P11	26.7	0	0
C8P12	86.7	46.7	26.7
C8P13	66.7	0	0
C8P14	26.7	13.3	33.3
C8P15	33.3	0	0
C8P16	80	0	0
C8P17	66.7	33.3	46.7
C8P18	13.3	0	0
C8P19	40	0	0
C8P20	46.7	0	0
C9P1	86.7	6.7	0
C9P2	93.3	6.7	0
Samba Mashuri 2 + Sub 1	100	0	0
Swarna + Sub 1	0	0	0
Korgbati	46.7	0	6.7
Galawah	93.3	0	20
Mean	57.29	6.36	8.58
SE Mean	3.61	1.66	1.9

3.1: AEROBIC AND ANAEROBIC GERMINATION PERCENTAGES AT 20 CM AND 10 CM WATER DEPTHS OF F3-F4 BREEDING LINES.

In aerobic germination, there were significant differences in germination percentages among breeding lines. In aerobic germination, line C6P11 has the highest germination percentage (100%) followed by lines C6P9, C6P10 and C9P2 with a germination percentage (93.3%) which is very close to the best check variety(Samba Mashuri 2 + SUB 1). In anaerobic germination under 20cm and 10 cm water depths, Line C6P11 scored the highest germination percentage at both levels followed by line C8P12, C6p10, C7P6, C8P17, C6P12, and C8P9 (Table 2) .

Table 3: Regeneration percentages of breeding lines at 20cm and 10cm water depths at 10 days after desubmergence

Line code	Regeneration % (20cm water depth)	Regeneration % (10cm water depth)
C1P1	0	13.3
C1P2	0	0
C1P3	6.7	0
C2P1	0	20
C2P2	6.7	13.3
C2P3	20	0
C5P1	0	13.3
C5P2	20	13.3
C5P3	33.3	46.7
C5P4	0	0
C5P5	0	26.7
C5P6	0	0
C5P7	6.7	0
C5P8	26.7	40
C5P9	33.3	46.7
C5P10	6.7	60
C5P11	6.7	0
C5P13	6.7	0
C6P1	26.7	26.7
C6P2	6.7	0
C6P3	20	0
C6P4	20	0
C6P6	20	0
C6P8	13.3	0
C6P9	20	33.3
C6P10	46.7	60
C6P11	93.3	86.7
C6P12	33.3	60
C7P1	6.7	0
C7P2	0	0
C7P3	6.7	0
C7P4	6.7	20

C7P5	20	26.7
C7P6	40	53.3
C7P7	6.7	6.7
C7P8	0	20
C7P9	40	53.3
C7P10	13.3	13.3
C8P1	26.7	53.3
C8P2	0	33.3
C8P3	20	26.7
C8P4	6.7	40
C8P5	6.7	13.3
C8P6	13.3	20
C8P7	0	13.3
C8P8	6.7	13.3
C8P9	26.7	53.3
C8P10	40	60
C8P11	6.7	6.7
C8P12	46.7	60
C8P13	20	26.7
C8P14	20	20
C8P15	26.7	40
C8P16	6.7	13.3
C8P17	46.7	66.7
C8P18	26.7	46.7
C8P19	6.7	0
C8P20	13.3	6.7
C9P1	40	53.3
C9P2	13.3	20
Samba Mashuri 2 + Sub 1	13.3	0
Swarna + Sub 1	40	46.7
Korgbati	6.7	13.3
Galawah	46.7	60
Mean	17.83	24.49
SE Mean	2.15	2.89

3.2: REGENERATION PERCENTAGES OF F3-F4 BREEDING LINES AFTER 10 DAYS OF DESUBMERGENCE AT 20CM AND 10 CM WATER DEPTHS

After 14 days of submergence at both 20cm and 10 cm water depth, breeding lines were desubmerged and allowed to regenerate for 10 days. The regeneration results at 20cm water depth showed that, there were significant difference existing among the breeding lines and also when compared to the best check (Galawah). Forty nine breeding lines regenerated under this condition. There was a significant difference between Line C6p11which scored the highest regeneration percentage and the best local check (Galawah). Forty eight breeding lines did not have any significant differences when compared to the best local check. Eleven breeding lines did not regenerate under this condition. In a similar view, four breeding lines can be grouped as high regenerators (Lines C6P11, C6P10, C8P12 and C8P17) with regeneration percentages ranging between 93.3% and 46.7%.

Observing the regeneration percentages of breeding lines at the 10cm water depth, 43 breeding lines regenerated. Line C6p11 had the highest regeneration percentage (86.7%), followed by line C8P17 (66.7%). These two lines had significant differences when compared with the best local check (Galawah). Forty-one breeding lines did not have any significant differences when compared with the best check under that condition. In general, Fifteen breeding (C6P11, C8P17, C6P10, C6P12, C5P10, C5P3, C8P12, C8P10, C5P9, C7P6, C7P9, C8P1, C8P9, C8P18 and C9P1) could be grouped as high regenerators with regeneration percentages ranging between 86.7% and 46.7%. (Table 3).

TABLE 4: MeanLeaf chlorophyll concentration percentages of Aerobic and Anaerobic germination at 20cm and 10cm water depths at 24 days after sowing.

Line code	% Chlorophyll concentration- Aerobic 1st set	% Chlorophyll concentration Aerobic 2nd set	% Chlorophyll concentration (Anaerobic -20cm water depth)	% Chlorophy concentration (Anaerobic -10c water depth)
C1P1	27.9	29.2	0	22.3

C1P2	27.4	0	0	0
C1P3	0	0	8	0
C2P1	26.9	28.4	0	24.7
C2P2	10.9	19.5	2.7	17.6
C2P3	17.1	15.1	3.7	0
C5P1	18.1	16.8	6.7	13.2
C5P2	16.6	14.6	2.6	12.9
C5P3	12.7	18.1	10.5	15.2
C5P4	19.2	22.1	0	0
C5P5	13.5	15.7	0	14.1
C5P6	17.5	0	0	0
C5P7	23.6	0	4.8	0
C5P8	18.9	20.1	14.7	17.3
C5P9	23.1	21.4	11.8	19.8
C5P10	26.6	25.6	10.6	18.9
C5P11	24.6	26.8	4.7	0
C5P13	27.6	25.3	3.9	0
C6P1	24.9	21.2	17.9	17.8
C6P2	9.4	14.9	2.6	0
C6P3	8.2	0	3.2	0
C6P4	15.8	14.6	2.5	0
C6P6	19.5	0	3.6	0
C6P8	14.5	0	12.1	0
C6P9	19.2	16.3	14.4	15.1
C6P10	29.6	24.5	12.1	21.4
C6P11	22.6	21.2	19.1	19.8
C6P12	13.4	12.7	11.8	11.4
C7P1	10.9	0	7.4	0
C7P2	11.4	0	0	0
C7P3	17.6	0	15.7	0
C7P4	13.1	16.2	3.5	10.5
C7P5	10	17.4	9.3	15.9
C7P6	16.3	18.3	10.1	16.2
C7P7	12.9	0	11 .2	11.6
C7P8	17.7	19.2	0	18.1

C7P9	21.5	18.5	15.9	16.6
C7P10	15.4	16.8	12.1	14.8
C8P1	21.3	19.6	8.2	18.1
C8P2	13.4	15.4	0	13.5
C8P3	15.7	17.2	9.5	15.6
C8P4	11.5	14.3	8.5	12.4
C8P5	18.7	15.6	10.8	14
C8P6	14.1	18.1	11.6	17.6
C8P7	18.9	19.7	0	16.8
C8P8	21.5	20.4	18.5	18.8
C8P9	20.9	18.9	19.2	17.1
C8P10	16.1	14.2	10.7	12.3
C8P11	16.7	18.2	10.5	17.6
C8P12	19.4	17.6	11.1	15.8
C8P13	18.8	20.3	8.9	19.4
C8P14	17.4	16.8	7.4	15.8
C8P15	18.8	21.4	12.1	20.2
C8P16	12.1	14.3	6.3	13.1
C8P17	18	20.1	14.1	16.7
C8P18	18.5	**17.6**	11.2	16.2
C8P19	15.5	0	4.7	0
C8P20	16.4	18.2	7.3	16.5
C9P1	18.2	0	12.8	14.8
C9P2	23.3	17.9	10.5	15.5
Samba Mashuri 2 + Sub 1	16	18.3	11.1	0
Swarna + Sub 1	0	0	18.3	15.1
Korgbati	20.4	16.4	14.4	15.2
Galawah	17.4	18.2	13.3	16.1
Mean	17.423	14.67	8.284	11.709
SE Mean	0.725	1.06	0.708	0.973

3.3: LEAF CHLOROPHYLL CONCENTRATION PERCENTAGES OF F3-F4 BREEDING LINES UNDER AEROBIC AND ANAEROBIC GERMINATION AT 20CM AND 10 CM WATER DEPTHS.

Under aerobic condition (sets 1 and 2) **,** leaf chlorophyll concentration percentages were measured after 24 days from the topmost leaves of five plants using an Electronic SPAD 502 meter and averaged. In aerobic set 1, there were significant differences in mean leaf chlorophyll concentration among the breeding lines and also when they were compared with the best check (Korgbati). The mean leaf chlorophyll percentages of 59 breeding were measured. Fifteen breeding lines, which included lines C1P1, C1P2, C2P1, C5P7, C5P9, C5P10, C5P11, C5P13, C6P1, C6P10, C6P11, C7P9, C8P1, C8P8 and C9P2 had mean leaf chlorophyll percentages ranging between 21.5% and 27.9% with significant differences compared to the best check. (Table 4).

In aerobic set 2, there were significant differences among the breeding lines when compared with the best check variety (Samba Mashuri 2 + SUB 1). Generally, Forty-seven breeding lines were measured for leaf chlorophyll concentration percentage. Nineteen of these lines had high significant differences in leaf chlorophyll concentration percentage ranging between 19.5% and 29.2 % when compared to the best check (Samba Mashuri 2 + SUB1) which had a percentage of 18.3% under this condition. Generally, eighteen breeding lines (C1P1, C2P1, C2P2, C5P4, C5P8, C5P9, C5P10, C5P11, C5P13, C6P1, C6P10, C6P11, C8P1, C8P7, C8P8, C8P13, C8P15 and C8P17) were grouped under high mean leaf chlorophyll percentage category. (Table 4).

In the anaerobic germination of F3-F4 breeding lines at 20cm and 10cm water depths, the average mean leaf chlorophyll concentration percentages were obtained from five plants after ten days of desubmergence. Breeding lines having below five plants, average mean leaf chlorophyll concentration percentages were obtained from existing plants, also after 10 days of desubmergence.

In anaerobic germination at 20cm water depth, there were significant differences in the mean chlorophyll concentration percentages among fifty breeding lines measured out of sixty. This indicates that ten breeding lines failed under this condition. Out of the fifty breeding lines, the mean chlorophyll concentration percentages of two breeding lines (C8P9 and C6P11) had high significant differences when compared with the best improved check (Swarna + SUB1). Their mean leaf chlorophyll concentration percentages ranged between 19.1 % and 19.2 %.

In a similar way, there were significant differences among breeding lines in anaerobic germination at 10cm water depth. However, 43 breeding lines were assessed while 17 failed under this condition. Out of the 43 germinated breeding lines at 10cm water depth, 17 had highly significant differences when compared with the best check variety (Galawah). These lines can be categorised as high mean leaf chlorophyll percentage category comprising of lines C1P1, C2P1, C2P2, C5P8, C5P9, C5P10, C6P1, C6P10, C6P11, C7P8, C8P1, C8P6, C8P8, C8P9, C8P11, C8P13 and C8P15 with percentages ranging between 17.1% and 24.7%. (Table 4).

Table 5: Mean Plant heights of Aerobic and Anaerobic germination at 20cm and 10 cm water depth at 24 days after sowing.

Line code	Plant height (cm) Aerobic 1st set	Plant. height (cm) Aerobic 2nd set	Plant height (cm) Anaerobic - 20cm water depth	Plant height (c Anaerobic- 10cm water depth
C1P1	25.4	29.3	0	26.1
C1P2	24	0	0	0
C1P3	0	0	26.6	0
C2P1	23.5	25.7	0	22.8
C2P2	29.4	35.1	20.9	31.3
C2P3	31	16.8	16.2	0
C5P1	17.9	18.9	0	18.6
C5P2	21.8	21.5	15.9	20.9
C5P3	15.9	19.3	12.4	17.2
C5P4	22.4	21.2	0	0
C5P5	15.9	21	0	19.8
C5P6	27.4	0	0	0
C5P7	15	0	9.6	0

C5P8	21.1	25.1	13.2	23
C5P9	20	20.4	4.7	19.5
C5P10	20.4	22.3	14.8	21.2
C5P11	18.2	23	13.1	0
C5P13	22.3	19.8	18.2	0
C6P1	19.4	24.3	14.7	21.4
C6P2	22.8	22.1	8.3	0
C6P3	18	0	14.8	0
C6P4	24.3	26.1	7.8	0
C6P6	17.9	0	7.2	0
C6P8	16.2	0	14.9	0
C6P9	20	23.5	14.7	19.6
C6P10	24.1	26.4	21.2	25.1
C6P11	18.9	23.6	16.5	20.4
C6P12	13.5	19.4	12.7	16.8
C7P1	22.8	0	19.8	0
C7P2	15.5	0	0	0
C7P3	18.7	0	15.9	0
C7P4	21.5	25.6	24.8	21.9
C7P5	11.5	21	10.4	17.3
C7P6	21.9	28.4	22.9	26.4
C7P7	30	0	9	29.8
C7P8	23.4	29.6	0	26
C7P9	23.7	29.3	17.5	27.2
C7P10	20.9	28.1	13.9	25.4
C8P1	21.3	25.6	13	23.2
C8P2	24.6	27.6	0	26.9
C8P3	21.1	28.3	14.5	24.6
C8P4	21.9	29.1	16	26.3
C8P5	21.3	24.8	11.3	23.5
C8P6	18.5	22.9	16.2	21.2
C8P7	15.3	21.3	0	19.2
C8P8	22.2	22.8	20	21.8
C8P9	19.6	20.4	19.1	19.8
C8P10	19.3	25.1	14.1	20.7

C8P11	18.1	22.3	20.1	18.6
C8P12	23.8	30.4	23.2	26.5
C8P13	18.4	26.1	15.9	19.1
C8P14	16.8	21.5	14.9	19.3
C8P15	20.7	29.2	7	27.3
C8P16	18.4	19.7	13.7	18.2
C8P17	20.2	20.8	15	19.4
C8P18	16.4	21.2	16.3	18.7
C8P19	22.8	0	20.2	0
C8P20	16.5	23.4	14.4	19.6
C9P1	8.7	0	9.7	15.4
C9P2	18.5	18.1	13.3	21.3
Samba Mashuri 2 + Sub 1	15.1	20.4	5.8	0
Swarna + Sub 1	0	0	11.6	21
Korgbati	19.8	26.2	14.9	21.6
Galawah	15.8	19.7	15	17.8
Mean	19.558	18.65	12.309	15.76
SE Mean	0.674	1.31	0.887	1.3

3.4: PLANT HEIGHTS OF F3-F4 BREEDING LINES UNDER AEROBIC GERMINATION AND ANAEROBIC GERMINATION AT 20CM AND 10CM WATER DEPTHS.

Plant height of F3-F4 breeding lines were measured using a ruler graduated in centimetres. The heights of five plants were measured and averaged for lines having more than five plants and for lines less than five plants, the height of all plants were measured and averaged at 24 days after sowing.

In the Aerobic germination set 1, there were highly significant differences in mean plant height among fifty-nine breeding lines. Twenty-nine of these breeding lines had highly significant differences when compared with the best check (korgbati) under this condition. Their mean plant heights ranged from 21.1cm to 29.4cm.

In the Aerobic set 2, there were significant differences in plant heights among 47 breeding lines. The plant heights of 11 breeding lines had highly significant differences when compared with the best check (Korgbati). Their mean plant heights ranged between 27.6cm and 35.1cm (Table 5).

In a similar way, there were significant differences in mean plant height among 49 breeding lines that produced seedlings under anaerobic germination at 20cm water depth. The mean plant heights of 20 of these breeding lines had highly significant differences; with mean plant heights ranging from 15.9cm to 26.6cm compared with the mean plant height (15cm) of the best check variety (Galawah). In the 2nd set of anaerobic germination at 10cm water depth, there were significant differences in the mean plant heights among forty-three breeding lines that germinated and grew into the seedling stage. Out of these forty-three breeding lines, the mean plant heights of 17 breeding lines had high significant differences compared with the best check (Korgbati) under this condition. Their mean plant heights ranged from 22.8cm to 31.3cm. (Table 5).

4.0: DISCUSSION

Rice is the most important cereal crop in the world consumed by nearly 3 billion people almost on a daily basis (Huke and Huke. 1997, Ito et al., 1999). In Sierra Leone, rice is our staple food grown in all the five agro-ecologies found in the country. Four of these agro-ecologies are lowland rice growing ecologies (Inland valley swamps, Mangrove swamps, Bolilands and Riverain grasslands) that are annually affected by varying degrees of submergence during the main cropping season-wet season (May to October).

In agreement to the above statement, the impacts of these floods seem to be worsening in recent years in the world with specific reference to Sierra Leone. These effects were commonly attributed to Climate Change such as Sea level rise, uneven distribution of rains, periodic changes in frequencies and intensities of flood caused by extreme weather events (Coumon and Rahmstorf, 2012).

Flood or Deep water research has been neglected for quite a long period by the Sierra Leone Agricultural Research institutes. In an attempt to mitigate this problem, crosses were carried out at the Rokupr Agricultural Research Centre (RARC) in 2014 to develop appropriate rice varieties that will combine high yields with submergence-tolerant ability. These observations were in line with Xu and Mackill (1996), who stated that, one of the most promising solutions is to develop high yielding varieties that are submergence-tolerant and more likely to be rapidly adopted by farmers in the target region.

Sixty breeding lines obtained from crosses done at RARC were screened for adaptability to flooded water condition during germination. This work is important because lowland rice farmers especially those working in Riverain grass lands and Bolilands normally suffer huge losses due to flooding of their rice fields sometimes immediately after sowing. This work is in agreement with work done at IRRIby screening of more than 8000 gene bank accessions and breeding lines (Ismail et al. 2009; Angajiet al. 2010). These accessions were phenotyped by sowing dry seeds in soil and then flooding with 80–100 mm (8cm- 10cm) of water.Angajiet al. (2009) also reported that tolerance to flooding during germination seemed relatively rare in rice. After screening over 8000 gene bank accessions, elite breeding lines and genotypes, they identified few genotypes with greater ability to germinate under flooding condition. In a similar way Ling et al. (2004) evaluated 359 accessions from different sources, including indica and japonica accessions, using a water depth of 20 cm at 30 °C.

Differences among breeding lines were demonstrated by the differences in their germination percentages at both aerobic and anaerobic conditions. In general, there was reduction in number of breeding lines that germinated and also germination percentages when comparing aerobic germination set 1 and set 2, thus indicating a reduction in the viability of F3-F4 seeds. In combining both aerobic germination sets 1 and 2, seven breeding lines have the highest germination percentages ranging between 80% and 93.3%. These included lines C6P11, C5P3, C6P9, C8P1, C8P2, C8P9 and C8P17.

Under the anaerobic germination condition at 20cm water depth, line C6P11 had the highest germination percentage (73.3%), followed by line C8P12 (46.7%). In a similar way under anaerobic germination at 10cm water depth, line C6P11 also scored the highest (66.7%) followed by lines C6P10 and C8P17. This was consistent with the findings of Jaing et al., (2006)who reported very limited success from previous efforts to improve the tolerance of genotypes for anaerobic conditions during germination. Angaji et al., (2009) also reported that, tolerance to flooding during germination seemed relatively rare in rice after screening 8000 gene bank accessions. They were able to identify few genotypes (about 0.23%) with greater ability to germinate under flooding condition. In another subsequent evaluation in replicated trials, there was further reduction in the number of accessions (about 0.06 %) that germinated under flooding condition (Jiang et al., 2006).

Breeding lines could be grouped into the following; those that germinated during 14 days of submergence and continue to grow after desubmergence, those that did not germinate during the 14 days of submergence but initiated germination after desubmergence. This was also observed by Winkelet al. (2013).Rice genotypes adapted to this type of flooding usually stay dormant or ‘quiescent’ when flooded to conserve their energy reserves and maintain their chlorophyll and underwater photosynthesis.

Besides other traits that confer tolerance to flooded water conditions, high regeneration ability of rice genotypes after flooded water recedes is an essential trait that contributes to high and stable productivity in flood-prone areas. This work is consistent with work done at IRRI, (2006). In this work, regeneration percentage results at 20cm water depth, showed that twenty-two breeding lines that germinated during the 14 days of submergence, 14 breeding lines fell under this group of those that germinated and resumed normal growth and increase in germination after desubmergence. Line C6P11 scored the highest regeneration percentage of 93.3%, followed by lines C6 P10, C8P12 and C8P17 scoring 46.7 %. Line C6P11 had high significant difference with the best check variety (Galawah) which scored 46.7%. Other lines in this category included lines C5P3, C5P2, C6P1, C6P12, C7P5, C7P6, C7P9, C8P10, C8P14, C9P1 and C9P2 with regeneration percentages ranging between 13.3% and 33.3% with no significant difference when compared with the best check variety. Five out of the remaining eight lines showed no increase in germination while three lines showed a decrease in germination. Twenty-nine breeding lines remained dormant (zero germination) at 14 days of submergence and germinated only after desubmergence. Under this category, lines C5P9, C5P8, C8P1, C8P15 and C8P18 had the highest regeneration percentage ranging between 33.3% and 26.7%.

In anaerobic germination and regeneration percentages at 10cm water depth, twenty-one lines germinated at 14 days after submergence and nineteen of these lines showed an increase in regeneration percentages at 10 days after desubmergence. The highest in this category is line C6P11 with a regeneration percentage of 86.7%, followed by lines C8P17, C6P10, C6P12, C8P10, C8P12, C7P6, C7P9, C8P1, C8P9 and C9P1 with regeneration percentages ranging between 53.3% and 66.7%. Seven out of the remaining nine breeding lines with increase in regeneration percentage had regeneration percentages ranging from 13.3% to 33.3%. Twenty-two breeding lines recorded zero germination (remained dormant) at 14 days of submergence but regenerated at 10 days after desubmergence. Amongst this category, line C5P10 had the highest regeneration percentage of 60%, followed by lines C9P1, C8P18, C8P15 and C8P4 with regeneration percentages ranging between 53.3% and 40.0%.

Chlorophyll concentration is an important trait in submergence-tolerant genotypes. A similar result was reported by Ella and Ismail (2006), Fukao et al. (2006) which shows that the ability of the tolerant genotypes to maintain higher chlorophyll during submergence, coupled with the positive correlations of survival with post-submergence leaf chlorophyll concentration, is an indication that, chlorophyll retention during and after submergence is critical for survival since it ensures underwater photosynthesis and faster recovery after the water recedes. In this work, Line C6P11 was among breeding lines that measured high mean leaf chlorophyll concentration percentages in both aerobic germination sets 1 and 2. Line C6P11 also maintained this trend for mean leaf chlorophyll concentration percentage in the anaerobic germination at both 20cm and 10cm water depths. Line C6P11 mean leaf chlorophyll percentage showed highly significant differences compared with the best check variety in anaerobic germination at 20cm and 10cm.

Plant height is also an important trait of submergence-tolerant rice genotypes that gives an indication of shoot elongation rate or fast recovery rate. This is consistent with work done by Takaiet al. (2010) wherein the height of 20 plants in each plot during the vegetative stage (from 10 to 50 DAT) was measured weekly to determine the shoot elongation rate. Under control conditions (Aerobic germination sets 1 and 2), plant height of breeding lines was statistically similar to that of the check varieties. In submerged treatments (anaerobic germination at 20cm and 10 cm water depths), tolerant breeding lines showed faster recovery after de-submergence with small differences in height when compared to some of the check varieties. Korgbati (Local Check variety) was slightly taller in height than Lines C6P11 and C8P12.

5.0: CONCLUSIONS AND RECOMMENDATIONS

5.1: CONCLUSIONS

- Since the opening of the Rice Research station Rokupr in 1934, now renamed as Rokupr Agricultural Research Centre (RARC), deep water or flooded water condition research in terms of developing adaptable genotypes has been neglected or not given due attention for such agro-ecologies that comprise of predominantly fertile soils and freshwater resources, and have enormous potential for enhancing food production to help meet the ever-increasing demands for rice. Two varieties (Gatang and Indochin Blanc) have been released so far but they were lost during the almost 11years (1991-2002) civil strife. There are no improved varieties developed for flood-prone ecologies. Therefore, screening of F3-F4 breeding lines for such ecologies where direct seeding method is mostly predominant among farmers was justified.

- Differences among breeding lines in germination percentage under anaerobic condition were demonstrated by some breeding lines showing more tolerance to standing water or flooded condition. It is on this basis that F3-F4 breeding lines were classified into those that can show appreciable germination percentage under anaerobic condition with limited shoot elongation.The most tolerant genotypes under this category also had the slowest underwater elongation. (Lines C6P11, C6P10, C8P17 and C8P12). The other category includes those that do not germinate (remain dormant or quiescent) during anaerobic condition but resume germination when optimum conditions for germination are available. The best lines in this category are C5P10, C8P18, and C9P1. There is another category of breeding lines that completely failed to germinate under anaerobic condition (during submergence) and after desubmergence, thus suggesting that direct screening and selection of breeding lines under anaerobic condition is essential to identify tolerant or adaptable lines to flooded or standing water condition.
- Adaptable breeding lines to germination under anaerobic condition demonstrated slight differences in Height and Chlorophyll concentration in their top most leaves when compared to breeding lines subjected to Aerobic germination (optimum germination conditions) and the best check varieties. This indicates fast recovery ability of tolerant breeding lines. This character could be used as an index for selecting tolerant or adaptable breeding lines to flooded or standing water condition.
- Breeding lines with good adaptability to flooded or standing water conditions exhibited slow or limited shoot elongation under water and exhibited fast recovery ability in plant height and high leaf chlorophyll percentage which functionally related and are important determinants of yield in adaptable genotypes. Developing high-yielding, stress-tolerant varieties is thus a strategic imperative that aims to provide farmers with a cost-effective option in flood-affected areas.

5.2. RECOMMENDATIONS

The experiment should be repeated in replicated trials in screen houses and thereafter in field where flooded and standing water condition stress is prominent before concrete conclusions could be drawn.

ACKNOWLEDGEMENT

The authors are grateful to Sierra Leone Agricultural Research Institute (SLARI) and Rokupr Agricultural Research Centre (RARC) for providing the laboratoryto carry out this research on rice. Appreciation is expressed to our colleagues Crop Improvement programme (Plant Breeding Division) in RARC for their support and valuable suggestions**.**

REFERENCES

1. **Angaji, S.A., E.M. Septiningsih, D.J. Mackill and A.M. Ismail (2009)**. QTLs associated with tolerance of flooding during germination in rice (Oryza sativa L.). Euphytica, 172: 159-168.
2. **Angaji, S., Septiningsih, E.M., Mackill, D.J., and Ismail, A.M (2010**). QTLS associated with tolerance of anaerobic conditions during germination in rice (oryza sativa L.). Euphytica 172, 159-168.
3. **Catling D.(1992)**. Rice in deep water. London: MacMillan Press
4. **Coumou D., Rahmstorf S. (2012).** A decade of weather extremes. Nat. Clim. Change 2, 491–496
5. **Das KK, Panda D, Sarkar RK, Reddy JN, Ismail AM (2009)**. Submergence tolerance in relation to variable floodwater conditions in rice, Environmental and Experimental Botany, vol. 66 (pg. 425 - 434)
6. **Ella ES, Ismail AM (2006).** Seedling nutrient status before submergence affects survival after submergence in rice. Crop Science.46:1673–1681
7. **FAO (2011**): Food and Agricultural Organization of the United Nations (FAO)
8. **Fukao T, Xu K, Ronald PC, Bailey-Serres J (2006)**. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. The Plant Cell; 18:2021-2034.
9. **Huke R.E. and Huke E.H (199**7). Rice area by type of culture. South, Southeast,and East Asia. A revised and updated database. International Rice Research Institute, Los Baños, Philippines, p. 59,
10. **IRRI (International Rice Research institute), (2006)**. Breeding for submergence tolerance

11. **Ismail, A.M., Ella, E.S., Vergara, G.V. and Mackill, D.J (2009).** Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (oryza sativa). Ann.Bot. 103,197-209
12. **Ito, O., Ella, E., and Kawano, N (1999)**. Pysiological basis of Submergence in rainfed Lowland rice Ecosystem. Field Crops research. 64, 75-90.
13. **Jiang, L., S. Liu, M. Hou, J. Tang, L. Chen, H. Zhai and J. Wan (2006)**. Analysis of QTLs for seed low temperature germinability and anoxia germinability in rice (Oryza sativa L.). Field Crop Res., 98: 68-75.
14. **Ling J, Ming-Yu H, Chun-Ming .W, Jian-Min W (2004)**. Quantitative trait loci and epistatic analysis of seed anoxia germinability in rice (Oryza sativa), Rice Science , vol. 11 (pg. 238-244)
15. **Mackill DJ, Ismail AM, Singh US, Labios RV, Paris TR, 2012**. Development and rapid adoption of submergence-tolerant (Sub1) rice varieties, Advances in Agronomy , vol. 115 (pg. 303-356)

16. **Takai T, Kondo M, Yano M, Yamamoto T (2010)**. A quantitative trait locus for chlorophyll content and its association with leaf photosynthesis in rice. Rice. 3:172–180.
17. **Winkel A, Colmer TD, Ismail AM, Pedersen O. (2013)**. Internal aeration of paddy field rice (Oryza sativa) during complete submergence-importance of light and floodwater O_2. New Phytologist. 197:1193–1203
18. **Xu K, Mackill DJ (1996).** A major locus for submergence tolerance mapped on rice chromosome 9. Molecular Breeding;2:219–224

19. **Yamauchi M, Aguilar AM, Vaughan DA, Seshu DV (1993)**. Rice (Oryza sativa L.) germplasm suitable for direct sowing under flooded soil surface, Euphytica , 1993, vol. 67 (pg. 177-184)