Research Article

Performance Evaluation of *Sub1* Rice Genotypes for Vegetative Stage Submergence Stress and Reproductive Stage Drought Stress

Ifrah Amjad[1], Muhammad Nouman Khalid[1*], Muhammad Kashif[1], Muhammad Noman[1], Sajid Ali[2], Rizwan Ahmed Shaikh[3], Muhammad Babar[4], Muhammad Asim Bhutta[5] and Amna Bibi[5]

[1]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan; [2]Soil and Water Testing Lab Toba Tek Singh, Punjab- Pakistan; [3]Department of Horticulture, Sindh Agriculture University Tandojam, Pakistan; [4]Department of Agriculture, University of Swabi, Khyber Pakhtunkhwa, Pakistan; [5]Cotton Research Institute, Multan, Pakistan.

Abstract | The present study was conducted to evaluate the genotypes having *Sub1* gene under submergence and drought stress in field conditions at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. A pot experiment was also performed to study the influence of complete submergence on elongation and survival percentage of five*Sub1* genotypes along with two high yielding local cultivars. All *Sub1* genotypes showed less elongation percentages than Super Basmati and KSK-133 while maximum survival percentage was observed in IR-07-F289 *Sub1* followed by Swarna *Sub1* and FR-13A. Five rice genotypes (FR-13A, Swarna *Sub1*, Ciherang *Sub1*, IR-07-F289 *Sub1* and IR-44 *Sub1*) along with two high yielding local varieties (KSK-133 and Super Basmati) were evaluated under submergence stress in field conditions. The field experiments were laid out in split plot randomized complete block design (RCBD) with three replications. Results of ANOVA revealed that chlorophyll contents, plant height, number of productive tillers per plant, panicle length, total spikelets per panicle, number of grains per panicle and 1000 grain weight were affected by submergence stress. While in another experiment drought stress was applied for 30 days on five *Sub1* genotypes along with Nagina-22 (Drought tolerant check) and IR-64 (drought susceptible check) in split plot design with three replications. Drought stress severely reduced all the parameters under study except leaf area, number of productive tillers per plant and biological yield per plant which remain unaffected. Overall results revealed FR-13A only produced grains under submergence stress while under normal and drought conditions it did not produced grains. All *Sub1* genotypes performed well under submergence stress. Swarna *Sub1* significantly produced more primary branches per panicle (10.5), yield (5.13 g) and harvest index (13.09) under drought stress as compare to the Nagina-22. Whereas remaining all *Sub1* genotypes also showed better performance than drought susceptible check (IR-64) and showed non-significant difference with Nagina-22 for most of the drought tolerance related traits. The results suggested that genotype having *Sub1* genes can effectively be grown under rainfed region which are equally prone to floods and drought stress.

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***Correspondence** | Muhammad Nouman Khalid, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan; **Email:** noumankhalidpbg@gmail.com
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Introduction

Rice (*Oryza sativa* L.) is widely cultivated around the globe extending from 50° north to 35° south. However, it is most susceptible crop to almost 42 different biotic (like bacterial leaf blight, sheath blight, blast and brown spot etc.) and abiotic stresses like drought, salinity, high and low temperatures etc. (Bhutta *et al*., 2019). It is typically cultivated in partial flooding conditions (Barik *et al*., 2019). However, flash floods severely damage the standing crop as entire plant becomes completely submerged in water (Woolston, 2014). The rice plant elongates its leaves and stems during flooding so as to evade submergence (Mori *et al*., 2019). Varieties of deepwater rice can do that quickly to survive (Akhter *et al*., 2019). Most of the rice cultivars die within seven to fourteen days of complete submergence by damaging cell membrane system, increasing biofilm fluidity, intensifying anaerobic respiration and changing metabolic balance, eventually leading to metabolic abnormalities (Xiong *et al*., 2019). Flash floods also cause significant crop damage at germination level and early seedling level leading to poor plant standing (Mahmood *et al*., 2019). It is frequent in uneven irrigated land and flood-prone rainfed ecosystems in a condition where rainfall occurs shortly after the seeding of rice (Tiwari, 2018). Yield loses due to flash floods depends on depth, duration, temperature and turbidity of flood water, soil fertility, fertilizer, seedling density, and age of the crop (Afrin *et al*., 2018).

Drought is another important problem in rice production (Mohanty *et al*., 2013). Drought stress not only reduces the grain yield but also affect the grain quality drastically. It reduces the dry matter accumulation in all plant organs and shortens the life cycle of the plant. It can occur at any growth stage of the crop (Upadhyaya and Panda, 2019). At vegetative stage drought stress reduces the growth of photosynthetic and storage organ of plant. Reproductive stage is most critical stage to drought stress (Hazman *et al*., 2019). At the time of flowering, it may limit the viability of pollen grains, receptivity of stigmas and seed setting (Korres *et al*., 2017). Thus, drought stress at reproductive stage affects the process of grain development resulting into spikelet sterility which ultimately reduces the yield (Swain *et al*., 2017; Bhutta *et al*., 2019). Submergence and drought stress can also occur successively within one season (submergence followed by drought and vice versa). An example happened in Luzon, Philippines, in 2006. During wet-season crop, seasonal rainfall surpassed 1,000 mm and within the same season a short spell of drought at flowering stage caused a dramatic decline in grain yield and harvest index (Mohanty *et al*., 2013).

To cope up the abrupt changes in climate, there is a dire need to develop high yielding cultivars which can tolerate multiple abiotic stresses (Arif *et al*., 2019). For this purpose, many scientists studied the genetic mechanisms involved in tolerance to submergence and drought stress. Submergence tolerance in rice is mainly controlled by a single locus present on chromosome 9 carrying cluster of three genes (*Sub 1*, *Sub1B* and *Sub1C*) which encode ethylene-response factors and are activated under flooding conditions (Septiningsih *et al*., 2015; Azarin *et al*., 2017; Dixit *et al*., 2017). Reports have shown that *Sub1* gene has pleiotropic effects on submergence and drought (Xiong *et al*., 2019). Rice genotypes having *Sub1* overcome this inevitable stress following de-submergence because it plays a pivotal role in detoxification of reactive oxygen species and stress inducible gene expression during drought (Fukao *et al*., 2011). These genotypes become able to form new leaves after stress. Thus, *Sub1* not only provides robust submergence tolerance but also improves survival of rapid dehydration following de-submergence and water deficit during drought (Bin-Rahman and Zhang, 2016). The present study was conducted to identify the genotypes having *Sub1* gene showing tolerance to not only the submergence stress but also the drought stress under field conditions in rice.

Materials and Methods

Experimental site and plant materials

The experiment was conducted in the fields of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during kharif season 2019. The plant materials was collected from Plant Breeding and Genetics Division (PBGD) which was comprised of five genotypes having sub-1 gene (FR-13A, Swarna-Sub1, IR-44-Sub1, IR-07-F289-Sub1, Ciherang-Sub1), two high yielding local cultivars (KSK-133, Super Basmati) and drought tolerant and drought susceptible checks (Nagina-22 and IR-64, respectively) (Mathan *et al*., 2021). The field experiments were laid out in split plot design with three replications. The genotypes were taken as sub plot factor while stress was taken as main plot factors. The row to row and plant × plant spacing was 9 inches. Gap-filling was also practiced as required after transplanting in order to ensure 100% plant establishment. All the necessary agronomic practices were performed during plant growth in normal and submerged conditions. Seeds were sown on the wet raised beds and 35 days seedlings were transplanted to well puddled soil.

Experiment 1: Survivability and shoot elongation rate of rice genotypes under complete submergence

Five *Sub1* genotypes along with two high yielding local cultivars (KSK-133 and Super Basmati) were evaluated under complete submergence stress. Three seedlings were transplanted in each pot. The pots were filled with dried soil from the paddy fields. Then, puddling of soil was done by adding water. Thirty-five days old seedlings were transplanted in the pots (3 plants per pot). The experiment was laid out in split plot design with three replications. After transplanting, 2 grams of urea + DAP were added in each pot. The seedlings were allowed to recover from transplanting shock for ten days. After ten days of transplanting, the pots were submerged completely in an outdoor concrete tank for 14 days. The water used for flooding was static, clean tube well water. No water changes were done during the stress conditions. After de-submergence, plants were allowed to recover for seven days and then data related to number of plants survived were recorded. Plant height of each genotype was also measured one day before submergence and one day after de-submergence.

Observations

- Elongation (per day) = (Plant height after de-submergence – Plant height before submergence)/ No. of days submerged.
- Elongation % = {(Plant height after de-submergence – Plant height before submergence)/ Plant height before submergence.} ×100
- Survival % = {(No. of plants before submergence – No. of dead plants)/No. of plants before submergence} ×100

Experiment 2: Evaluation of rice genotype under complete submergence in field conditions

A field experiment was conducted to evaluate the impact of submergence on yield and yield contributing traits of rice. For this purpose, five sub-1 genotypes along with two high yielding local cultivars (KSK-133 and Super Basmati) were evaluated under field conditions. All genotypes were evaluated in a natural water pond for a period of 21 days. The transplanted seedlings were allowed to establish their roots and to recover from transplanting shock for 20 days before submergence. Then, seedlings were completely submerged by filling the pond with normal canal water for 21 days. The stress was maintained by adding water at daily basis in the pond to overcome the water loss due to percolations or evaporation. The cutting of leaves of plant above the water surface was also done twice in whole stress period to ensure the complete submergence of plant. After completing 21 days of complete submergence, the stress was terminated by draining water out of the pond.

Experiment 3: Evaluation of rice genotype under drought in field conditions

A field experiment was carried out to test the hypothesis that genotypes having *Sub-1* gene can also survive during drought stress. Five *Sub1* genotypes were evaluated. Nagina-22 and IR-64 were grown as tolerant and sensitive checks respectively, as both genotypes were frequently used in the past as checks during screening and also in numerous morpho-physiological studies. The drought stress conditions were made by stopping normal irrigation at booting stage for 30 days.

Observations recorded

The chlorophyll content was measured by the SPAD-502 Plus and leaf area was measured by portable leaf area meter AM300 at the heading stage of the plants. At the time of harvesting, plant height (cm), productive tillers per plant, primary branches per panicle, filled spikelets per panicle, unfilled spikelets per panicle, fertility percentage, panicle length (cm), biological yield per plant (g), grain yield per plant (g) and harvest index were measured.

Statistical analysis

The data of both submergence and drought stress experiments was analyzed by analysis of variances (Steel *et al*., 1997; Ostertagova and Ostertag, 2013), Tuckey mean comparison tests (Tuckey, 1949) and Dunnett multiple comparison test (Dunnett, 1955) by Statistix 8.1.

Results and Discussion

Survivability and shoot elongation rate of rice genotypes under complete submergence

Shoot elongation rate (elongation per day) of different genotypes under submergence stress was ranged from 0.12 cm to 1.44 cm. Minimum elongation per day was observed in Ciherang *Sub1* (0.12 cm) followed by IR-44 *Sub1* (0.18cm) and IR-07-F289 *Sub1* (0.182cm). Highest elongation per day under complete submergence stress was exhibited by KSK-133 (1.44cm) and Super Basmati (1.27cm). Submergence stress enhanced the plant height of all genotypes ranging from 7.32% to 83.94%. The height of *Sub1* genotypes increased within the range of 7.32% (exhibited by Ciherang*Sub1*) to 53.25% (exhibited by FR-13-A *Sub1*). While KSK-133 and Super Basmati exhibited 83.94% and 77%, respectively (Table 1). Percentage survival of all genotypes upon de-submergence was varied from 33.33% (observed in Ciherang*Sub1*) to 77.78% (exhibited by IR-07-F289-*Sub1*). Survival percentage of KSK-133 was 55.56% and of Super basmati was 44.44% (Table 1).

Analysis of variance and mean performance of genotypes for yield and yield related traits under submergence

Results of ANOVA under submergence stress in field conditions revealed that chlorophyll contents, plant height, number of productive tillers per plant, panicle length, total spikelets per panicle and number of grains per panicle showed significant results. Whereas, leaf area, number of primary branches per panicle, number of sterile spikelets per panicle, spikelet fertility percentage, biological yield per plant and grain yield per plant were not significantly affected by submergence stress. The genotypes × treatment interaction was also highly significant for plant height, total spikelets per panicle, number of grains per panicle number of sterile spikelets per panicle, spikelet fertility %, gain yield per plant, harvest index and 1000 grain weight (g) revealing that the joint effect of genotypes and treatment levels on these traits were higher than their individual effects (Table 2).

Post hoc test (Table 3) revealed that the mean values for chlorophyll contents under submergence stress were greater than the normal conditions (Table 3). The chlorophyll contents were ranged from 39.11 (observed in KSK-133) to 42.81 (observed in Super Basmati) under submergence. While under normal conditions, maximum chlorophyll contents were observed in Swarna *Sub1* (44.45) followed by KSK-133 (40.36) and IR-07-F289 *Sub1* (39.77). Leaf area was ranged from 2726 m^2 to 3843.33 m^2 under submergence stress and 2895.66 m^2 to 4335 m^2 under normal conditions. Maximum plant height was observed in Super Basmati (121.04 cm) followed by KSK-133 (117.89 cm) and minimum plant height was observed in Swarna *Sub1* (75.91 cm). When the mean values of all the genotypes for number of productive tillers were compared, it was revealed that highly significant results were due to super basmati which was significantly different from KSK-133 and Ciherang *Sub1*. Panicle length was ranged from 21.3 cm to 25.77 cm under normal conditions while 21.38 cm to 24.94 cm under submergence stress. The significance of genotypes primary branches per panicle was only due to the Swarna *Sub1*. Mean values of number of grains per panicle were ranged from 85.06 to 184.26 under normal conditions while 102.11 to 126.88 under submergence stress. Maximum number of sterile spikelets were observed in Swarna *Sub1* (28.88) followed by IRR-44 *Sub1* (20.44) and Super Basmati (12.11) while minimum mean values were observed for IR-07-F289-*Sub1* (4.77). Mean values of fertility % were varied from 79.08 % to 96.74 % under normal conditions while from 81.45 % to 95.53 % under submergence conditions. Post hoc test also revealed that the all the genotypes under study had grain yield different from one another. Swarna *Sub1* exhibited highest grain yield (25.64g) under stress followed by Ciherang *Sub1* (24.13g). Under complete submergence stress, maximum value for harvest index was observed in Super Basmati (42.92 g) and minimum value was observed in IR-07-F289 *Sub1* (34.64 g).

Table 1: *Elongation per day, elongation % and survival % of rice genotype under complete submergence.*

S. No.	Varieties	Plant height before stress (cm)	Plant height after stress (cm)	Elongation per day (cm)	Elongation %	Survival %
1	FR-13A *Sub 1*	32.3	49.5	0.95	53.25	55.56
2	Swarna *Sub 1*	22.57	26.5	0.21	17.41	66.67
3	IR-44 *Sub 1*	26.94	30.33	0.18	12.58	33.33
4	IR-07-F289 *Sub 1*	30.04	44.83	0.182	49.23	77.78
5	Ciherang *Sub 1*	30.43	32.66	0.123	7.32	33.33
6	KSK-133	30.77	56.6	1.44	83.94	55.56
7	Super Basmati	29.66	52.5	1.27	77	44.44

Table 2: *Analysis of variance table for some important yield related traits under submergence stress.*

Sources of variation	DF	Chlorophyll contents	Leaf area (cm^2)	Plant height (cm)	Productive tillers per plant	Panicle length (cm)	Primary branches per panicle	Total spikelets per panicle	Number of grains per panicle	Number of sterile spikelets per panicle	Spikelet fertility %	Biological yield per plant (g)	Gain yield per plant (g)	Harvest Index	1000 grain weight (g)
Replication (R)	2	8.07	403100	12.82	0.42	0.48	0.87	0.32	8.19	11.12	11.33	53.49	2.56	62.72	3.84
Treatment (T)	1	78.08**	120756	773.71*	492.59**	5.09*	0.01	2838.5**	1575.69*	184.49	11.53	6.96	96.51	355.29	133.78*
Error R×T	2	1.41	190219	18.32	1.21	0.11**	0.07	11.83	30.04	23.12	9.92	228.09	8.36	29.23	4.8
Genotype (G)	5	16.61	1277616*	1545.06**	14.68**	11.27	10.08*	2389.78**	493.62**	907.36*	335.41**	45.15	102.96**	457.84*	48.1**
T×G	5	29.67	583249	103.61*	1.76	2.21	1.18	956.46**	556.35**	92.49**	20.17*	126.37	32.28*	159.6**	5.51**
Error R×T×G	20	11.15	492047	25.21	1.24	2.12	1.63	41.31	44.54	10.33	5.96	178.12	9.89	65.37	5.21
Total	35	15.75	584285714	273.81	16.84	3.32	2.59	583.42	222.65	155.96	55.75	142.57	28.35	140.97	14.96
CV(R×T)		2.87	12.02	8.13	12.10	1.41	2.85	26.47	12.03	18.96	5.16	28.31	10.42	14.05	15.07
CV(R×T×G)		8.06	19.34	7.82	17.29	6.32	13.08	35.12	23.19	25.67	6.29	25.02	18.26	21.84	18.9

** = at 0.05 level of significance, ** = at 0.0 level of significance.*

Table 3: *Tukey HSD All-Pairwise comparisons test of some yield related traits for treatments and genotypes under submergence.*

	Chlorophyll contents	Leaf area (cm^2)	Plant height (cm)	Productive tillers per plant	Panicle length (cm)	Primary branches per panicle	Total spikelets per panicle	Number of grains per panicle	Number of sterile spikelets per panicle	Panicle fertility %	Biological yield per plant (g)	Gain yield per plant (g)	Harvest Index	1000 grain weight (g)
Treatments (Main plot factors)														
Submergence	42.93 a	3684.9 a	106.96 a	7.31 b	23.44 a	9.73 a	129.56 a	112.74 a	14.31 a	88.21 a	53.78 a	20.45 a	38.02 a	24.95 a
Control	39.98 b	3569.1 a	97.69 b	15.1 a	22.69 b	9.77 a	111.81 b	99.51 b	12.30 a	90.01 a	53.39 a	23.72 a	44.42 a	21.1 b
Genotypes (Sub plot factors)														
Swarna *Sub1*	43.37 a	3772.5 ab	74.52 d	11.45 bc	22.49 b	13.01a	155.82 a	120.43 a	35.38 a	77.38 d	54.55 a	19.23 cd	35.25 bc	18.26 c
IR-44 *Sub1*	41.39 a	2810.8 b	102.79 bc	12.35 ab	23.36 ab	8.67 b	99.92 d	95.44 c	4.48 d	95.5 a	56.12 a	25.29 ab	45.06 abc	25.1 ab
IR-07-F289 *Sub1*	40.41 a	4146 a	94.54 c	10.94 bc	21.93 b	9.96 b	131.08 b	108.24 b	22.83 b	83.21 c	55.11 a	16.13 d	29.26 c	23.5 ab
Ciherang*Sub1*	43.61 a	3671.3 ab	111.98 a	9.9 c	23.66 ab	9.33 b	112.09 c	106.53 bc	5.55 d	95.07 a	49.77 a	22.14 abc	44.44 ab	24.4 ab
Super Basmati	40.22 a	3904.7 ab	119.34 a	13.63 a	25.35 a	9.19 b	109.99 cd	97.17 bc	12.82 c	88.25 b	54.48 a	27.73 a	50.89 a	21.1 bc
KSK-133	39.74 a	3456.8 ab	110.76 ab	10.02 c	21.61 b	9.36 b	115.21 c	108.91 ab	6.3 d	94.59 a	49.99 a	22.01 bc	44.02 ab	25.6 a

Means within each column followed by the same letter are not significantly different from each other at 0.05 level of significance.

Analysis of variance and mean performance of genotypes for yield and yield related traits under drought

Results of ANOVA revealed that drought stress severely reduced the chlorophyll contents, plant height, panicle length, number of primary branches per panicle, total spikelets per panicle, number of grains per panicle, number of sterile spikelets per panicle, spikelet fertility percentage, grain yield per plant and harvest index. While leaf area, number of productive tillers per plant and biological yield per plant remain unaffected (Table 4). The genotypes × treatment interaction was also highly significant for all traits under study except panicle length, number of grains per panicle and biological yield per plant (Table 4).

Tuckey post hoc test showed that for chlorophyll contents, variation among genotypes came from three homogeneous groups (a, b and ab) which were significantly different from each other (Table 5). Plant height under drought stress was ranged from 50.65 cm to 81.88 cm while under normal conditions it ranged from 73.13 cm to 143.73 cm. Post hoc test revealed that all genotypes were divided into 4 groups (a, b ab, and c) for productive tillers per plant. Maximum panicle length was observed in IR-64 (31.42 cm) followed by Ciherang *Sub1* (22.84 cm) under normal conditions. While under stress conditions panicle lengths varied from 15.41 cm to 25.4 cm. When post hoc test was applied for primary branches per panicle, it revealed that the significant results of genotypes were only due to Swarna *Sub1* because this was the only genotype which was significantly different from other genotypes for primary branches per panicle. Under drought stress maximum sterile spikelets were produced by susceptible check IR-64 (101.83). The above ground biomass was varied from 48.8 g to 118.93 g under normal conditions and 29.66 g to 112.93 g under drought stress. Under normal conditions grain yield per plant was ranged from 18.85 g (observed in Swarna *Sub1*) to 38.03 g (observed in Nagina-22) and under drought stress it varied from 0.98 g (exhibited by IR-44 *Sub1*) to 5.13 g (observed in Swarna *Sub1*). Maximum yield reduction was observed in IR-64 (-96.52 %) as it was taken as drought susceptible check. When means of genotypes compared, it revealed that genotypes were distributed among four groups for harvest index. Maximum harvest index was observed in Ciherang *Sub1* (51.18 g) followed by IR-44 *Sub1* (45.24 g) under normally irrigated conditions. While under drought, its values varied from 0.96 g to 13.94 g. Minimum decrease in harvest index under drought was observed in IR-07-F289 *Sub1* (-44.64 %) followed by Swarna *Sub1* (-64.15%).

Two-sided dunnett's multiple comparisons for yield and yield related traits under drought stress

Dunnett's multiple comparison test revealed chlorophyll contents of all genotypes were not significantly different from Nagina-22 (which was used as tolerant check) under drought conditions (Table 6). IR-07-F289 *Sub1* and IR-64 had significantly more leaf area than Nagina-22 under drought stress while IR-44 *Sub1* had significantly less leaf area than Nagina-22. The mean values of plant height of all *Sub1*genotypes were significantly less than nagina-22. While IR-64 had plant height comparable to nagina-22. IR-44 *Sub1* and Ciherang *Sub1* produced more number of productive tillers per plants as compare to Nagina-22, while the mean values of Swarna *Sub1* and IR-07 F289 *Sub1* were comparable to the mean value of Nagina-22. The susceptible check (IR-44) had lowest number of productive tillers per plant among all genotypes under drought. Panicle lengths of all *Sub1* genotypes were comparable to Nagina-22 because the differences were non-significant. Swarna *Sub1* exhibited more primary branches per panicle as compared to the Nagina-22 under drought conditions. The mean values of total number of spikelets per panicle under drought conditions for all genotypes were compared with the Nagina-22. The mean values of total numbers of spikelets per panicle of all *Sub1* genotypes showed non-significant differences when compared with Nagina-22. The susceptible check IR-64 had maximum number sterile of spikelets per panicle and also were higher than Nagina-22 under drought conditions (Table 6). Swarna *Sub1* and IR-07-F289 *Sub1* performed comparable to Nagina-22 under drought stress while Ciherang *Sub1* and IR-44 *Sub1* had significantly very low fertility percentage as compare to the Nagina-22. The mean values of all *Sub1* genotypes were significantly less than Nagina-22 for biological yield per plant. Swarna *Sub1* had more grain yield under drought conditions as compare to Nagina-22. While Ciherang*Sub1* had almost similar grain yield to the Nagina-22. Swarna *Sub1* and IR-07-F289 *Sub1* had significantly more harvest index than Nagina-22 under drought stress while Ciherang*Sub1* and IR-44 *Sub1* had comparable value to the Nagina-22.

Table 4: *Analysis of variance table for some important yield related traits under drought stress.*

Sources of variation	DF	Chlorophyll contents	Leaf area (m^2)	Plant height (cm)	Productive tillers per plant	Panicle length (cm)	Primary branches per panicle	Total spikelets per panicle	Number of grains per panicle	Number of sterile spikelets per panicle	Spikelet fertility %	Biological yield per plant	Gain yield per plant	Harvest Index
Replication (R)	2	21.11	273769	0.9	5.03	4.32	0.44	1622	2705.3	240.2	171	604.06	3.97	75.46
Treatment (T)	1	78.81*	1509622	14731.1**	22.56	245.86*	18.06*	21707.1*	64507.5**	11374.2*	14852.5*	1469.7	4521.20**	7916.52**
Error R×T	2	2.74	141718	52	10.65	2.085	0.61	949.3	410.3	371.9	264.4	135.82	4.38	12.48
Genotype (G)	5	43.45**	6962444**	1916.7**	78.37**	81.45**	7.73**	6801.6*	4112.9	1482.9**	1024.5**	7528.92**	98.63**	168.20**
T×G	5	52.01**	1319377**	462.3**	34.56**	5.83	2.64*	1619.6	3444.2	2073.1**	1767.2**	136.88	128.27**	246.39**
Error R×T×G	20	9.74	270124	14.7	3.38	4.17	0.74	1665.2	1906.6	272.4	167.2	80.22	0.86	15.52
Total	35	22.82	1.4E+09	772.1	19.61	22.24	2.48	2921.74	4190.2	1023.63	943.55	1225.22	162.55	299.31
CV(R×T)		4.56	10.12	8.62	22.97	6.78	8.39	25.47	21.06	26.11	23.32	16.97	14.98	16.52
CV(R×T×G)		8.61	13.97	4.58	12.95	9.60	9.29	33.73	15.11	12.30	18.54	13.04	6.63	18.42

** = at 0.05 level of significance; ** = at 0.0 level of significance.*

Table 5: *Tukey HSD All-Pairwise comparisons test of some yield related traits for treatments and genotypes under drought.*

	Chlorophyll contents	Leaf area (m^2)	Plant height (cm)	Productive tillers per plant	Panicle length (cm)	Total spikelets per panicle	Number of grains per panicle	Number of sterile spikelets per panicle	Spikelet fertility %	Primary branches per panicle	Biological yield per plant (g)	Gain yield per plant (g)	Harvest Index
Treatment													
Drought	34.78 b	3926.4 a	63.39 b	13.42 a	18.66 b	96.41 b	47.08 b	49.333 a	49.424 b	8.583 b	62.306 a	2.770 b	6.562 b
Normal	37.74 a	3516.8 a	103.85a	15 a	23.89 a	145.52 a	131.74 a	13.783 b	90.048 a	10.01 a	75.084 a	25.184 a	36.220 a
Genotypes													
Swarna *Sub1*	39.22 a	3684.7 bc	61.89 e	12.61 b	17.9 b	120.32 ab	90.32 a	30 ab	73.770 abc	11.517 a	45.88 b	11.993 cd	24.815 ab
IR-44-*Sub1*	35.41 ab	2587.2 d	76.50 d	17.72 a	21.02 b	79.53 b	45.62 a	33.92 ab	52.058 c	8.400 b	39.97 b	11.482 d	24.362 ab
IR-07-F289-*Sub1*	36.93 ab	4543.8 ab	71.03 d	11.67 bc	19.91 b	89.47 b	72.63 a	29ab	80.862 ab	9.117 b	48.86 b	9.720 e	19.569 bc
Ciherang*Sub1*	37.58 a	3273.3 cd	84.02 c	16.81 a	21.04 b	139.90 ab	104.48 a	35.42 ab	62.320 bc	8.733 b	49.04 b	13.602 c	28.019 a
Nagina-22	31.37 b	2832.2 cd	110.20a	17.4 a	19.41 b	124.77 ab	109.92 a	14.850 b	86.885 a	8.700 b	112.48 a	21.009 a	17.989 bc
IR-64	37.05 a	5408.5 a	98.08 b	9 c	28.42 a	171.82 a	113.48 a	58.33 a	62.521 bc	9.283 b	115.93 a	16.056 b	13.594 c

Means within each column followed by the same letter are not significantly different from each other at 0.05 level of significance.

Table 6: *Two-sided dunnett's multiple comparisons for yield and yield related traits under drought.*

Genotypes	Nagina-22	Swarna *Sub1*	IR-44-*Sub1*	IR-07-F289-*Sub1*	Ciherang *Sub1*	IR-64
Chlorophyll contents	33.24	34	34.18	34.08	34.11	39.06
Leaf area (m^2)	3564.7	3867.7	2278.7-*	4752.7*	2952	6142.7*
Plant height (cm)	76.66	50.65-*	56.75-*	58.31-*	56-*	81.66
Productive tillers per plant	13.66	10.5	18.83*	9.33	19.5*	8.66-*
Panicle length (cm)	15.42	14.5	19.21	18.26	19.2	25.42*
Primary branches per panicle	7.33	10.5*	8.66	8.83	7.33	8.83
Total spikelets per panicle	87.33	97.17	69.67	78	89	157.30*
Number of sterile spikelets per panicle	109.92	90.32	45.62	72.63	104.48	113.48
Spikelet fertility %	17.17	30	63.5	19	64.5	101.83*
Primary branches per panicle	81.44	68.55	8.93-*	76.01	28.65-*	32.94-*
Biological yield per plant (g)	106.57	39.63-*	29.67-*	35.83-*	49.20-*	112.93
Gain yield per plant (g)	3.17	5.13*	0.98-*	3.93	2.31	1.07-*
Harvest index	3.02	13.09*	3.48	13.94*	4.85	0.96

**Indicates a significant difference from Nagina-22 at 95% probability level.*

Global climate change has an impact on the frequency and magnitude of hydrological fluctuations, which can result in catastrophic events such as floods and droughts, among other things. Extremes in precipitation, both high and low, are rapidly limiting food, fiber, and forest production across the planet (Mohanty *et al*., 2013). As a result, improving rice's combined resistance to submergence and drought will significantly enhance rice yield while also preserving water resources and soil quality (Xiong *et al*., 2019). In order to do this, genotypes containing the submergence resistant gene (*Sub1*) were assessed in fields under total submergence and severe drought conditions for yield-related characteristics.

In pot experiment highest elongation per day and stem elongation percentage under complete submergence stress was exhibited by KSK-133 and Super Basmati. All the *Sub 1* genotypes showed less elongation rate and elongation percentage than local cultivars not having *Sub1* gene same results were also observed by Sarkar and Bhattacharjee (2011), Akinwale *et al*. (2012) and Sarkar *et al*. (2014), Panda and Sarkar (2012) and Yadav *et al*. (2018) observed that that survival %age was negatively correlated with plant elongation under submergence. But an extreme reduction in elongation percentage also caused death of plants. So, overall results of present study indicated that extreme reduction in elongation of shoot during stress is not always directly related to the survival of the plants upon de-submergence as KSK-133 showed highest elongation percentage but it also showed good survival percentage comparable to FR-13-A which is considered as submergence tolerant genotype (Sarkar *et al*., 2014; Sevanthi *et al*., 2019).

All the genotypes that maintain high chlorophyll contents during submergence and post-submergence period are considered as tolerant genotypes (Singh *et al*., 2015). So, all the genotypes in present study maintained similar chlorophyll contents under submergence as well as under normal conditions. Winkel *et al*. (2014) observed that *Sub1* was involved in chlorophyll protection during submergence stress. So, all *Sub1* genotypes had higher mean values of chlorophyll contents under submergence and control conditions. These results proved that all genotypes under study maintained higher chlorophyll contents after de-submergence and can be categorized as submergence tolerant genotypes.

Complete submergence stress always induces an increase in plant height in rice. So, all the genotypes under study had more plant heights under submergence stress as compare to the normal conditions. Plant height was more in KSK-133 and Super Basmati as compare to *Sub1* genotypes. Sultana *et al*. (2018) observed the increase in plant heights in all genotypes but elogation percentage was less in tolerant genotypes as compared to the susceptible ones. Because, all *Sub1* genotypes can survive very well under complete submergence conditions and give good yields. So, in this study submergence stress did not significantly affect the grain, yield, biological yield and harvest index. Same trend was observed by Panda and Sarkar (2012) when they evaluated Swarna and Swarna *Sub1* under complete submergence. They observed that the introgression of *Sub1* into popular varieties did not have any apparent negative effects on grain yield, yield attributes, and harvest index under controlled field conditions, but considerably enhanced grain yield following submergence. They also observed that the biological yield of the *Sub1* genotypes under submergence stress was similar to their biological yield under control conditions. The same trend was also reflected in harvest index for all the genotypes. But in this study, KSK-133 and Super Basmati which did not have *Sub1* gene also performed well under stress conditions because of the fact that they might have developed the tolerance mechanism for complete submergence as these local cultivars face flood every year.

Under drought stress the chlorophyll contents were significantly reduced due to severe drought stress as compared to the control conditions. Singh *et al.* (2018) and Mishra *et al.* (2018) also observed a severe decline in chlorophyll contents of rice plant under drought stress. Drought stress did not affect the leaf area and number of productive tillers per plant because drought stress was applied at booting stage of the plant. So, plants had already developed their panicle. Mishra and Chaturvedi (2018) also observed a severe decline in panicle lengths of the plants when stress was applied on booting stage. Number of grains per spike and spikelet fertility % and grain yield per plant were severely affected by drought as stress was applied at reproductive stage. Haque *et al.* (2016) also observed that drought stress at reproductive stage significantly reduced the number of grains per panicle due to which grain yield was decreased by 39%. Akram *et al.* (2013) also observed the severe drought stress increased the spikelet sterility %. He and Serraj (2012) also reported that terminal drought stress reduced the water potential in leaves and panicles and also reduced the spikelet fertility by 64.6% as compared to control. Swarna *Sub1* produced significantly more yield and harvest index under drought stress as compare to the Nagina-22.

Conclusions and Recommendations

Improvement of combined tolerance to submergence and drought would substantially increase rice productivity. Results showed that all *Sub1* genotypes performed well not only under submergence stress but also under drought stress. Swarna *Sub1* significantly produced more primary branches per panicle yield and harvest index under drought stress as compare to the Nagina-22. Whereas remaining all *Sub1* genotypes also showed better performance than drought susceptible check (IR-64) and showed non-significant difference with Nagina-22 for most of the drought tolerance related traits. The results recommended that genotype having *Sub1* genes can effectively be grown under rainfed region which are equally prone to floods and drought stress.

Novelty Statement

Previous studies have shown that Sub1 gene not only provides tolerance to submergence stress but also plays a functional role in survival of plants after drought. But all these studies were conducted in laboratory conditions. The present study was conducted to test this hypothesis exclusively in field conditions.

Author's Contribution

Ifrah Amjad: Conducted the research and wrote-up the manuscript.
Muhammad Nouman Khalid and Rizwan Ahmed Shaikh: Helped in manuscript write-up
Muhammad Kashif: Supervised the study.
Muhammad Noman: Helped in data collection.
Sajid Ali: Proof read the manuscript.
Muhammad Babar and Amna Bibi: Helped in data analysis.
Muhammad Asim Bhutta: Provided technical guidance during research work.

Conflict of interest
The authors have declared no conflict of interest.

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