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SUBMERGENCE AND DROUGHT STRESSES IN RICE OVER GENOTYPE BY ENVIRONMENT INTERACTION

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SUMMARY

Freshwater swamplands are potential areas for rice cultivation. The obstacle to rice cultivation in freshwater swampland is water stress, i.e., submergence stress at vegetative stage and drought stress at the reproductive stage. Both stresses may occur during the same planting season. This study aimed to evaluate genotype by environment interactions and the tolerance of 12 rice genotypes in a normal environment and a double-stress (submergence + drought) environment. The experiment was conducted at the ICRR experimental station in Sukamandi, Subang, West Java, under different environmental conditions: (1) normal condition at an irrigated lowland field and (2) double-stress condition in a submergence pool, wherein submergence stress was provided at vegetative stage and drought stress was provided at reproductive stage. Five lines, seven cultivars, and a randomized complete block design with three replications were used in each environment. Results showed that IR11T210 was the most stable genotype in both environments and was therefore considered tolerant to double stresses. IR11T210 had a yield of 7.30 t/ha under normal condition and 5.58 t/ha under double-stress condition with a yield reduction of 23.6%. Under double-stress conditions, this line had a plant height of 95.03 cm and 30 tillers, which were equivalent to the plant height and tiller number of Inpari 30 Ciherang Sub1, the best check cultivar. This line was expected to satisfy farmer’s needs.

Keywords: Double stresses, freshwater swampland, genotype by environment interaction, rice, tolerance

Key findings: IR11T210 was identified as tolerant to double stresses, specifically, submergence at vegetative stage and drought at reproductive stage. This study provides information on the yield and yield component traits of 12 rice genotypes under double stresses. This information can be useful in rice breeding programs.

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INTRODUCTION

The need for rice as a staple food has increased in line with population growth. Given that the global population is expected to increase to 9.2 billion in 2050, agricultural productivity must increase by 60% relative to agricultural productivity in 2005–2007 (Fita *et al.*, 2015). The conversion of land use from agricultural to nonagricultural is also a limiting factor for increasing lowland rice production. Suboptimal land can be used as a buffer for rice production. Freshwater swamplands are among suboptimal land types with the potential for development.

In Indonesia, freshwater swampland (*lebak*) covers an area of approximately 13.3 million ha across the islands of Sumatra, Kalimantan, and Papua. The largest area of freshwater swampland, reaching 2.98 million ha, is located in the Province of South Sumatra (Subagyo, 2006). Water management is the main obstacle to rice cultivation in freshwater swampland is given that during the rainy season, the entire area is submerged for long periods; this condition cannot be controlled (Gusmiatun *et al.*, 2015). By using a groundwater level graph, Waluyo *et al.* (2008) observed that rice cultivation in freshwater swampland, especially shallow areas, often experience two stresses during one planting season: submergence stress during the vegetative stage (April–May) and drought stress during the reproductive stage (August).

The type of submergence that occurs in shallow freshwater swampland is full submergence, which is also referred to as complete submergence. Flash floods generally occur for 1–2 weeks (Mackill *et al.*, 1993; Nugraha *et al.*, 2013). Young rice plants are usually highly sensitive to submergence stress (Jackson and Ram, 2003). Submergence stress inhibits photosynthesis and respiration as a result of the reduction in the intensity and speed of gas diffusion; gas diffusion in water is 104 times slower than that in air (Ikhwani *et al.*, 2010). The Directorate of Food Crop Protection (2010) reported that during the 2009/2010 rainy season in Indonesia, flooding struck 12 provinces, causing crop failure.

Kurniawati *et al.* (2014) stated that drought is a major environmental factor that affects the growth and stability of crop production. Drought stress has a considerable effect on plant growth, although the range of reduction varies considerably due to differences in the time and intensity of stress imposition and the cultivars used (Emam *et al.*, 2010). Drought during the generative phase (terminal drought) reduces the availability of groundwater to plants progressively and may cause plant death. The

Table 1. Genetic material used in this study.

S.No.	Cultivars	Status
1	IR11T210	Promising line
2	IR96321-1447-651-B-1-1-2	Promising line
3	B13926E-KA-1	Promising line
4	IR96321-1447-521-B-2-1-2	Promising line
5	BP20452e-PWK-0-SKI-2-3	Promising line
A	Inpari 30	Tolerant check for submergence
B	IR42	Sensitive check for submergence
C	Limboto	Tolerant check for drought
D	IR20	Sensitive check for drought
G	Inpara 8	Swampland cultivar
I	Inpari 38	Rainfed lowland cultivar
J	Inpari 9	Irrigated lowland cultivar

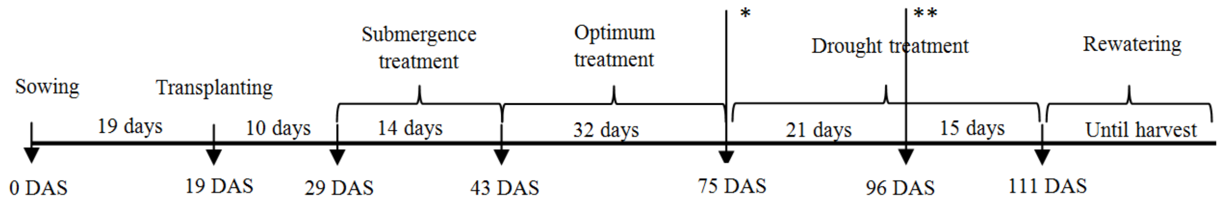
capability of a genotype to last longer and maintain function under drought conditions results in subsistence yield, which is considerably lower than the yield obtained under optimal conditions (Bhargava and Sawant, 2013). Drought tolerance allows plants to grow and maintain relatively high yields despite drought conditions and is a result of the capability of plants to withstand or recover from stress (Bhargava and Sawant, 2013).

In consideration of the effects of the double stresses that occur in shallow swamplands, we selected lines and cultivars that are tolerant of submergence and drought in separate experiments (Wening *et al.,* 2019a; Wening *et al.,* 2019b). Evaluating the responses of genotypes in a double-stress experiment is interesting. This study aimed to evaluate genotype by environment interaction (GEI) and the tolerance of several genotypes for the double stresses of submergence at vegetative stage and drought at reproductive stage.

MATERIALS AND METHODS

This experiment was conducted at Sukamandi Experiment Station, Subang, West Java, Indonesia, from June to November 2018. The experiment was carried out under two environmental conditions, i.e., normal and double-stress conditions. The double-stress condition was imposed in a submergence pool to simplify treatment, and normal condition was carried out in irrigated lowland. The experimental design was a randomized complete block design with three replications. The materials were 12 genotypes, including five previously selected promising lines (Wening *et al.,* 2019a; Wening *et al.,* 2019b) and seven check and popular cultivars (Table 1).

Seedlings aged 19 days after sowing (DAS) were transplanted with a plant spacing of 25 cm x 25 cm in a 50 cm x 500 cm plot area or adjusted to the availability of the seedlings. Submergence treatment was performed 10 days after planting (DAP) or 29 DAS with a water level of 75 cm above the ground. Submergence was stopped approximately after 14 days of submergence when IR42 showed sensitive symptoms. The plants were subsequently maintained normally until 75 DAS. Irrigation was then stopped until 111 DAS. At 96 DAS, the



Note : * = Stopped irrigation; ** = drought stress period (<−60 kPa); DAS = days after sowing.

Figure 1. Stress treatment on one planting season.

plants were subjected to severe drought of less than −60 kPA, and this treatment was maintained for 15 days (Figure 1). Rewatering was performed at 111 DAS until harvesting. According to Gu *et al.* (2013) −50+/−5 kilopascal soil moisture exerts severe drought stress on rice growth.

The observed variables included days to flowering, days to maturity, plant height, number of tillers, panicle length, number of filled grains per panicle, number of empty grains per panicle, panicle fertility, 1000-grain weight, and yield in stress and control plots. In the experiment with the double-stress treatment, the number of individuals and plant vigor were observed before submergence and 7 days after submergence treatment was stopped. During the drought period, leaf rolling score (0 = leaves healthy; 1 = leaves start to fold [shallow]; 3 = leaf folding [deep V-shape]; 5 = leaves fully cupped [U-shape]; 7 = leaf margins touching [O-shape]; 9 = leaves tightly rolled) and leaf drying score (0 = no symptom; 1 = slight tip drying; 3 = tip drying extended up to 1/4 length in most leaves; 5 = 1/4 to 1/2 of all leaves dried; 7 = more than 2/3 of all leaves fully dried; 9 = all plants apparently dead) were observed on the basis of the standard evaluation system (SES) (IRRI, 2014).

Yield (t/ha) in the control experiment was calculated by using the following formula:

$$Y = \frac{160{,}000}{no.of\ plants\ in\ a\ plot} x\ plot\ yield\ (kg) x \frac{100 - MC}{86} x1000,$$

where Y = yield (ton/ha); 160,000 = the number of plants in 1 ha with a spacing of 25 cm x 25 cm; MC = grain moisture content.

The same formula was used in the stress experiment, except the number of plants in a plot was changed to the initial population size.

Data were subjected to analysis of variance by using SAS. G x E interaction was evaluated through combined analysis across two environments. The method used to describe tolerance level was based on the decrement in the percentage of yield with the following equation:

$$\%\ decrease\ of\ yield = \frac{Yo - Ys}{Yo} x100,$$

where Yo = Yield under normal condition, and Ys = Yield under stress condition.

RESULTS AND DISCUSSION

General conditions of the experiment

Under normal treatment, plants grew well without any considerable presence of pests and diseases. Under double-stress treatment, crop performance was good and vigorous before submergence. Submergence was carried out for 14 days. Under submergence, all parts of the plant were immersed in water. Submergence treatment resulted in different responses among genotypes. Survival rate ranged from 2.29% to 79.02%. Five promising lines and one check cultivar (Inpari 30 Ciherang Sub1) had good survival rates (>50%). The survival rate of Inpara 8 was 16.09%, whereas those of other cultivars were less than 10%. Postsubmergence, the plants showed optimal cultivation, and all of the remaining plants grew well without plant pests and diseases. Drought-treated plants showed leaf drying symptoms with scores of 1 to 5. The check cultivars Inpari 30 Ciherang Sub1 and Inpara 8 showed good performance with drying scores of 3 and 1, respectively. The average number of harvested plants per plot ranged from 0 plants to 18 plants depending on tolerance to double stresses (Table 2).

After submergence, IR42, IR20, and Limboto had an average of 1 to 3 surviving plants. These genotypes were categorized as very sensitive to submergence stress. The remaining plants then died due to oxidative stress after water subsidence. Several studies have shown that plants can be tolerant when submerged but not tolerant when submergence recedes because of oxidative damage due to aerobic conditions. Rice plants must convert from anaerobic metabolism to aerobic metabolism when water recedes. The sensitive plants failed to adapt to postsubmergence aerobic conditions and experienced oxidative stress because of the low concentration of ascorbic acid postsubmergence (Kawano *et al.*, 2002).

Inpari 38 lacked tolerance to submergence stress as indicated by its low survival rate. It was the remaining plant that survived, and it had green plants in two replications. This cultivar was classified as rainfed lowland rice (Jamil *et al.*, 2016) and was thus tolerant of drought stress. In this experiment, Inpari 9 had the unique characteristic of sensitivity to submergence stress. Upon exposure to drought stress, its remaining plants could still grow but were unable to produce panicles. Inpari 9 could be categorized as sensitive to double stresses.

Effect of genotype, environment, and genotype by environment interaction on agronomic traits

Genotype, environment, and G × E interactions had significant effects on yield and different effects on morphological and agronomic characters varied. All genotypes were included in the analysis of variance of yield characters even though the sensitive genotypes mostly failed to reproduce. Only seven genotypes were included in the analysis of variance of morphological and agronomic characters given that sensitive plants mostly died. The effects of genotypes, environments, and G × E interactions on all of the traits can be seen (Table 3).

Table 2. Status of each genotype postsubmergence and drought in the experiment under double-stress treatment (submergence at vegetative stage and drought at reproductive stage).

S.No.	Lines / Cultivars	Status post-submergence (survival rate %)	Status post-drought (leaf drying score)	IP	SP	HP
1	IR11T210	Survived (56.41%)	3	23	13	15*
2	IR96321-1447-651-B-1-1-2	Survived (79.02%)	3	29	23	15
3	B13926E-KA-1	Survived (54.56%)	5	27	15	12
4	IR96321-1447-521-B-2-1-2	Survived (70.36%)	5	27	19	15
5	BP20452e-PWK-0-SKI-2-3	Survived (65.22%)	5	26	17	18*
A	Inpari 30	Survived (62.18%)	3	27	17	14
B	IR42	Died (2.29%)	-	28	1	0
C	Limboto	Died (8.62%)	3[a]	28	3	1
D	IR20	Died (8.97%)	-	29	3	0
G	Inpara 8	Survived (16.09%)	1	29	5	6*
I	Inpari 38	Died (2.34%)	7[b]	29	1	2
J	Inpari 9	Died (6.78%)	3[c]	20	1	0

Note: IP = number of initial plants; SP = number of surviving plants postsubmergence; HP = number of harvested plants; a = only replication 2; b = only replication 2 and 3; c = no panicle exertion; * = the number of plants harvested was greater than the number of plants that survived after submergence because some plants exhibited slow recovery.

Table 3. Effect of the genotype (G), environment (E), and G × E interaction.

Characters	G	E	G × E	CV (%)
Days to flowering	95.19**	5394.66**	56.66**	3.98
Days to maturity	30.3 ns	3547.52**	51.86*	3.70
Plant height	319.66**	56.24**	25.60**	2.061
Number of productive tillers	50.37 ns	142.27*	40.66 ns	29.81
Panicle length	2.99*	34.02**	2.50 ns	4.15
Number of filled grains	672.95*	13626.01**	267.11 ns	21.65
Number of unfilled grains	318.61 ns	1134.64 ns	235.52 ns	38.02
Number of total grains	673.88 ns	6909.47**	258.25 ns	14.95
Percentage of filled grains	219.40 ns	2733.15**	118.07 ns	22.17
Percentage of unfilled grains	219.40 ns	2733.15**	118.07 ns	29.66
1000 grain weight	41.67**	49.10**	5.56*	5.96
Yield	10.52**	437.79**	4.12**	16.88

Note: * = significant at the 5% level; ** = significant at the 1% level; ns = not significant; CV = coefficient of variability.

The agronomic performance and morphological characters of the genotypes showed responses to the double stresses. Submergence stress resulted in large spaces between individual plants because several plants died. The distance between plants and drought stress during the reproductive phase would affect the number of tillers, plant height, and yield components.

Analysis of variance showed that the environment influenced all characters except for the number of unfilled grains per panicle. Double stresses affected the total number of grains but did not affect the number of unfilled grains. This result showed that at the time of panicle initiation, double stresses affected the number of spikelets per panicle. Panicle initiation occurred when the plant experienced drought stress. Torres and Henry (2018) stated that drought reduces panicle fertility.

Double stresses affected plant phenology. The results of this study indicated that double stresses delayed days to flowering and maturity (Table 3). Yullianida *et al.* (2014) reported that submergence stress delays days to flowering and maturity. Torres and Henry (2018) also reported that drought delays days to flowering.

G × E interaction had significant effects on days to flowering, days to maturity, plant height, 1,000-grain weight, and yield. G × E interaction showed the differences in the rank of lines in both environments, in this case, between the double-stress environment and the normal environment. Akçura and Ceri (2011) stated that significant G × E interactions result in differences in responses between normal conditions and stress conditions.

G × E interaction and tolerance of plants to double stresses

One of the objectives of two-location experiments (two environments in this study) is to determine G × E interactions and the patterns of genotype responses to the environment (Mattjik and Sumertajaya, 2013). Tolerant genotypes showed the smallest reduction in yield (in normal vs. stress environment). Suhartina *et al.* (2014) defined stability as the capability of lines to avoid large yield changes in various environments. Components that cause a genotype to have stable yields include tolerance to stress (Purwantoro *et al.*, 2012) and speedy recovery after stress (Suhartina *et al.*, 2014). The percentage of yield reduction is widely used to determine the tolerance of a plant to stress (Riduan *et al.*, 2005; Mohammadi *et al.*, 2010; Zao *et al.,* 2019). In this study, Inpari 30 Ciherang Sub1 was the best check cultivar which exhibited less yield reduction than other cultivars (58.7%). Inpari 30 Ciherang Sub1 is a cultivar that contains the Sub1 gene. Fukao *et al.* (2011) reported that the presence of the Sub1A gene is not only important for resistance to submergence stress but is also associated with an increase in the capability of plants to avoid dehydration after submergence and water deficit during drought. This capability was thought to enable Inpari30 Ciherang Sub1 to continue providing high yield despite experiencing the double stresses of submergence and drought.

The results of this study indicated that three lines had lower yield decrements than Inpari 30 Ciherang Sub1. These lines were IR11T210, IR96321-1447-651-B-1-1-2, and BP20452e-PWK-0-SKI-2-3, which had yield reductions of 23.6%, 45.1%, and 45.3%, respectively. These three lines could be categorized as tolerant to double stresses. Adaptive genotypes could be identified on the basis of the yield obtained in each environment. The experiment under double-stress condition indicated that IR11T210 was the most adaptive line to double stresses with a yield of 5.58 tons/ha followed by BP20452e-PWK-0-SKI-2-3 with a yield of 4.41 tons/ha.

Table 4. Mean performance of genotypes for days to flowering, days to maturity, plant height, and number of tillers in each environment and delta of both conditions.

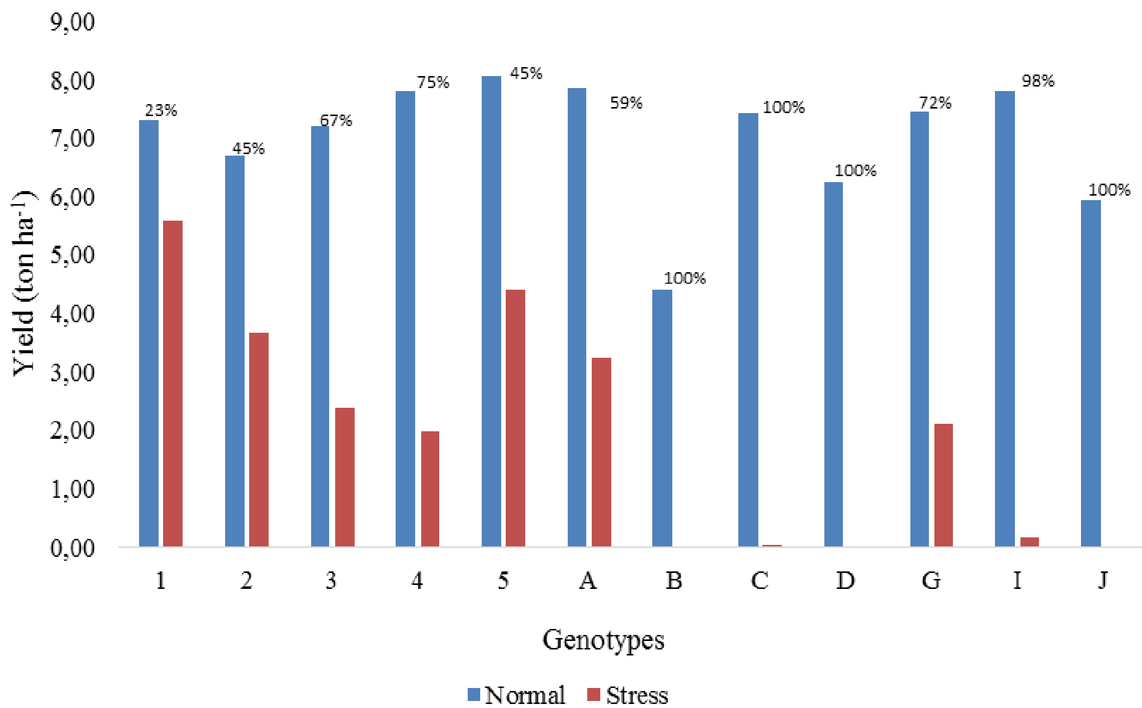
S.No.	Days to flowering			Days to maturity			Plant height			Number of tillers		
	Sub+dro	Normal	Delta	Sub+dro	Normal	Delta	Sub+dro	Normal	Delta	Sub+dro	Normal	Delta
1	98.6 c	80.3 b	−18.33	122.0 ab	101.0 b	−21.0	95.0 c	104.1 c	9.07	30.5 a	19.2 a	−11.3
2	106.6 b	92.3 a	−14.33	123.3 ab	112.3 a	−11.0	94.2 c	89.5 e	−4.70	18.8 a	19.2 a	0.3
3	107.3 b	84.6 ab	−22.67	122.0 ab	102.0 ab	−20.0	99.3 c	99.7 d	0.47	16.5 a	18.7 a	2.1
4	115.0 a	88.6 ab	−26.33	120.3 b	109.6 ab	−10.6	105.4 b	109.5 b	4.16	16.6 a	15.3 ab	−1.2
5	109.6 b	83.0 ab	−26.67	125.3 a	99.6 b	−25.6	109.1a b	110.9 ab	1.73	18.9 a	15.0 ab	−3.8
A	116.6 a	83.0 ab	−33.67	124.6 a	101.0 b	−23.6	100.1 c	102.9 cd	2.90	20.2 a	18.7 a	−1.5
G	105.3 b	88.6 ab	−16.67	123.3 ab	106.6 ab	−16.6	111.7 a	114.3 a	2.57	24.5 a	14.2 b	−10.3
Avg	108.5	85.8	−22.7	123.0	104.6	−18.4	102.1	104.4	2.3	20.9	17.2	−3.7

Note: 1 = IR11T210; 2 = IR96321-1447-651-B-1-1-2; 3 = B13926E-KA-1; 4 = IR96321-1447-521-B-2-1-2; 5 = BP20452e-PWK-0-SKI-2-3; A = Inpari 30 Ciherang Sub1; G = Inpara 8; Avg = average; the value followed by the same letter in the same column and treatment factor show no significant difference based on DMRT at 5% level; Sub+dro = submergence + drought stresses; Delta = difference between sub+ dro and normal.

Table 5. Mean performance of rice genotypes for filled grain per panicle, unfilled grain per panicle, total grain per panicle, panicle length, and 1000-grain weight in each environment and delta of both.

S.No.	Number of filled grains per panicle			Number of unfilled grains per panicle			Number of total grains per panicle			Panicle length			1,000-grain weight		
	Sub+ Dro	Normal	Delta	Sub+ dro	Normal	Delta	Sub+dro	Normal	Delta	Sub+ dro	Normal	Delta	Sub+ dro	Normal	Delta
1	39.9 b	90.6 a	50.8	47.7 a	35.7 d	−12.0	87.7 b	126.4 a	38.7	26.7 a	24.4 a	−2.3	20.9 bc	23.5 c	2.6
2	66.9 ab	100.4 a	33.4	61.7 a	48.9 bc	−12.8	128.7 a	149.3 a	20.6	23.9 c	24.3 a	0.4	22.1 bc	20.3 d	−1.9
3	45.7 b	91.2 a	45.4	70.0 a	41.7 bcd	−28.3	115.8 ab	132.9 a	17.1	24.5 c	23.2 a	−1.3	18.7 c	21.1 d	2.4
4	37.6 b	81.2 a	43.6	70.2 a	51.7 b	−18.5	107.8 ab	132.9 a	25.1	26.0 a	24.0 a	−2.9	23.4 ab	27.6 a	4.1
5	40.8 b	79.5 a	38.6	53.8 a	63.5 a	9.6	94.7 ab	142.9 a	48.3	26.9 a	23.5 a	−3.5	24.5 ab	27.4 a	2.9
A	55.9 ab	85.9 a	29.9	51.9 a	39.6 cd	−12.4	107.9 ab	125.5 a	17.6	25.0 bc	23.8 a	−1.3	22.5 ab	25.7 b	3.2
G	80.7 a	91.2 a	10.4	46.3 a	47.8 bc	1.5	127.0 ab	139.0 a	12.0	26.0 ab	24.5 a	−1.5	26.0 a	27.8 a	1.8
Avg	52.6	88.6	36.0	57.4a	47.0	-10.4	109.9 a	135.6a	25.6	25.7	23.9	-1.8	22.6	24.7	2.2

Note: 1 = IR11T210; 2 = IR96321-1447-651-B-1-1-2; 3 = B13926E-KA-1; 4 = IR96321-1447-521-B-2-1-2; 5 = BP20452e-PWK-0-SKI-2-3; A = Inpari 30 Ciherang Sub1; G = Inpara 8; Avg = average; the value followed by the same letter in the same column and treatment factor show no significant difference based on DMRT at 5% level; sub+dro = submergence + drought stresses; Delta = difference between sub+dro and normal.



Note: Value at the top of the bars is percentage of reduction; 1 = IR11T210; 2 = IR96321-1447-651-B-1-1-2; 3 = B13926E-KA-1; 4 = IR96321-1447-521-B-2 1-2; 5 = BP20452e-PWK-0-SKI-2-3; A = Inpari 30 Ciherang Sub1; B = IR42; C = Limboto; D = IR20; G = Inpara 8; I = Inpari 38; J = Inpari 9.

Figure 2. Grain yield of lines tested under normal conditions and double stresses, as well as the percentage decrease.

Morphological and agronomic characters of tested lines

The morphological and agronomic performance of the lines tested under double stresses and normal conditions are shown in Tables 4 and 5. Double-stress treatment extended days to flowering to 22 days and days to maturity to 18 days (Table 4). Double stresses also reduced the number of filled grains per panicle to 36 grains and increased the number of unfilled grains per panicle to 10 grains per panicle (Table 5). Plant height, panicle length, and 1000-grain weight under the double-stress treatment were not significantly different from those under normal treatment. The number of tillers per plant decreased to 3.7 (Table 4), thus reducing the competition between plants and enabling the formation of a large number of tillers after submergence. By contrast, the results of the drought experiment by Torres and Henry (2018) showed that drought reduces plant height but does not affect the number of tillers. Pandey and Shukla (2015) reported that drought reduces plant height, tiller number, and leaf number and size.

On the basis of the results of this study, the genotype with tolerance for double stresses, submergence at vegetative stage, and drought at reproductive stage was identified as IR11T210. Under stress conditions, this line had a plant height of 95.0 cm, number of tillers of 30, and 1000-grain weight of 20.9 g. IR11T210 had a yield of 7.3 ton/ha under optimal conditions and 5.58 t/ha under double-stress conditions with a yield reduction of 23.6% (Figure 2). Among the 12 evaluated genotypes, five cultivars, namely, IR42, IR20, Limboto, Inpari 38, and Inpari 9, were sensitive to double stresses. IR11T210 was tolerant to double stresses, submergence at vegetative stage, and drought at reproductive stage.

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