

Effects of Drought Stress on Growth and Yield of Rice (*Oryza sativa* L.) Cultivars and Accumulation of Proline and Soluble Sugars in Sheath and Blades of Their Different Ages Leaves

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Abstract: One of the main problems of rice cultivation and production is the lack of water resources, especially during periods of low rainfall which affect the vegetative growth rate and the amount of yield. In this study the effect of low water supply on the number of heading per hill, number of grain per hill, dry weight of vegetative tissues and panicle and 1000 grain weight in three new cultivars of rice including 216, 829 and Zayandeh-Rood were measured under submerged and non-submerged conditions in a randomized complete block design with three replicates. Simultaneously, the variation in proline and total sugars in sheaths and blades of leaves at different ages was determined. The data indicated that Zayandeh-Rood cultivar showed the lowest reduction in shoot dry weight and the number of tillers per hill under non-submerged conditions. Furthermore, the panicle weight and the number of filled grains per spike were higher in Zayandeh-Rood cultivar than the other cultivars. In addition, the result of this study shows that Zayandeh-Rood cultivar in which originated from local cultivars, have higher ability in solute accumulation such as proline and total carbohydrates than the other new lines. Due to correlation between drought tolerance of Zayandeh-Rood and solute accumulation, it may be suggested that the solute accumulation is one of the mechanisms for drought tolerance in rice.

Key word: Rice (*Oryza sativa* L.) • Drought stress • Proline • Carbohydrate • Yield components • Grain filling

INTRODUCTION

Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world [1, 2]. According to statistics, the percentage of drought affected land areas more than doubled from the 1970s to the early 2000s in the world [3]. Drought is a world-spread problem seriously influencing grain production and quality and with increasing population and global climate change making the situation more serious [4]. Rice (*Oryza sativa* L.) as a paddy field crop is particularly susceptible to water stress [5, 6]. It is estimated that 50% of the world rice production is affected more or less by drought [7].

To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. In addition to the complexity of drought itself [1, 2], plant's behavior responses to drought are complex and different mechanisms are adopted by plants when they

encounter drought [8, 9]. One mechanism utilized by the plants for overcome the water stress effects might be via accumulation of compatible osmolytes, such as proline [10, 11], soluble sugars [12]. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm. Thus, proline can be used as a metabolic marker in relation to stress [13]. Moreover, under drought stress the accumulation of total soluble sugars in different plant parts would be increased [14]. However the rate of additional production or accumulation of proline and soluble sugar is different in different plant parts. Although plant's leaf would monitor the osmolytes components more compared to the other plant parts, sheaths account for more contents rather than blades. Of course, the sensitivity of leaves toward drought stress depends on their ages and segments of plants [15, 16].

A rice leaf is composed of sheath, lamina joint and blade, acropetally in the longitudinal axis [17]. The leaf sheath is the main part of the plant that plays a major role

in transporting all essential elements with water from the roots to aerial portions of the plant. The leaf sheath supports and stiffens the plant against environmental conditions.

Drought stress during vegetative growth, flowering and terminal period of rice cultivation, can interrupt floret initiation (which cause spikelet sterility) and grain filling, respectively [18, 19]. On the other hand, it has been proposed that grain filling is closely linked to the whole-plant senescence process [20, 21]. Usually, water stress at grain filling process induces early senescence and shortens the grain filling period but increases remobilization of assimilates (are reserved in the stems and sheaths of rice and contribute 10-40% of the final grain weight) from the straw to the grains [22, 23]. Therefore, soil drying is unfavorable to plant growth but may not be unfavorable to production of grain if the plant is tolerant to drought stress and is capable to cope with the stress condition prior to grain filling process.

The present study was conducted to determine how drought affects grain yield and the process of grain filling in three new cultivars of rice and also test the hypothesis that differential drought tolerance in new rice seedlings was related to quantitative aspects of the leaf osmolytes status such as proline and soluble carbohydrates in the leaves.

MATERIALS AND METHODS

This experiment was conducted in late April 2005 in glass house of Biology Department, University of Isfahan, Iran. Three new cultivars of rice including 216, 829 and Zayande-Rood were planted in separated pans to have seedling. In May of 2005, three rice cultivars seedlings were transferred to 9 lysimeters (200×120×100 cm) according to a randomized complete block design with two treatments of submerged and aerated condition in three replicates. During the growing season, the water level kept up to 5 cm in submerged treatment but in non-submerged plots the water applied as closely spaced borders type irrigation. The amount of water consumption was recorded during growing season for different lysimeters. The soil texture and also some soil physical and chemical characteristics such as available N, P, K, organic carbon, pH and EC were analyzed prior to planting time.

The harvest time was nearly 150 days after cultivation. At this time the ratio of heading per plant and the number of total, filled and unfilled grains per hill were measured. The plant samples were separated in different

parts such as vegetative tissues, panicle and grains. Then, different plant parts were incubated at 70°C and their dry weights were calculated. Afterward, the amount of proline and total sugars in young and old leaves and also sheaths and blades were determined and compared in various ages (young and old leaves) and different parts (sheath and blade) in all three cultivars.

Determination of Proline and Soluble Sugars: Proline accumulation in fresh leaves was determined according to the method of Bates *et al.* [24]. Free proline was extracted from the leaves of plants using aqueous sulfosalicylic acid. The filtrate (1 ml) was mixed with equal volumes of glacial acetic acid and ninhydrin reagent (1.25 g ninhydrin, 30 ml of glacial acetic acid, 20 ml 6 NH₃PO₄) and incubated for 1 h at 100°C. The reaction was stopped by placing the test tubes in cold water. The samples were rigorously mixed with 3 ml toluene. The light absorption of toluene phase was estimated at 520 nm using Pharmacia LKB- Novaspec II model spectrophotometer. The proline concentration was determined using a standard curve. Free proline content was expressed as $\mu\text{mol lit}^{-1}$ of plant parts.

Determination of Soluble Sugars Content: Soluble sugars were determined based on the method of phenolsulfuric acid [25]. 0.1g dry weight of leaves was homogenized with deionized water, extract was filtered and the extract treated with 1% phenol and 98% sulfuric acid, mixture remained for 1h and then absorbance at 485 nm was determined by spectrophotometer (Pharmacia LKB- Novaspec II model). Contents of soluble sugar were determined by using glucose as standard and expressed as mg g^{-1} .

RESULTS

The results of soil analysis indicated that soil texture is clay loam included 47.7% gravel. The chemical soil analysis indicated that there were no noticeable differences in the amount of organic components and mineral nitrogen between submerged and non-submerged treatments. Moreover, in terms of electrical conductivity (EC) there is not also a considerable alteration in both treatments. However, pH has moderately decreased. The saturation percentage and CaCO₃ content of soil in all unit experiments remained constant. In contrast, the amount of available phosphorus and potassium under non-submerged treatment were increased up to 13.1% and 10.4%, respectively (Table 1).

Table 1: The mean values of soil chemical characteristics of the 9 lysimeters (Sp, saturated point; TNV, Total Neutralizing Value; OC, organic carbon; P and K, available P and K)

Lysimeter	EC(ds/m)	pH	SP(%)	TNV(%)	OC(%)	N(%)	P ppm	Kppm
Submerged	3.23	7.1	46.33	26.67	3.67	0.37	166.67	416.8
Non-submerged	3.38	7.4	45.50	25.50	3.66	0.37	191.67	438.3

Table 2: Proline content ($\mu\text{mol lit}^{-1}$) of sheath under submerged and non-submerged conditions

Rice cultivars	Young Leaves			Old Leaves		
	Submerged	Non-submerged	Change (%)	Submerged	Non-submerged	change (%)
Zayande-Rood	112	155	0.384	58	73	0.258
829	84	102	0.214	48	52	0.083
216	97	120	0.237	54	61	0.129
Average	97.67	125.67	0.286	53.3	62	0.163

Table 3: Proline content ($\mu\text{mol lit}^{-1}$) of blade under submerged and non-submerged conditions

Rice cultivars	Young Leaves			Old Leaves		
	Submerged	Non-submerged	Change (%)	Submerged	Non-submerged	Change (%)
Zayande-Rood	116	129	0.112	58	60	0.034
829	82	85	0.036	45	45	0.00
216	100	104	0.04	51	53	0.039
Average	99.3	106	0.067	51.3	52.7	0.026

Table 4: Total soluble sugar content (mg g^{-1} DW) of sheath under submerged and non-submerged conditions

Rice cultivars	Young Leaves			Old Leaves		
	Submerged	Non-submerged	Change (%)	Submerged	Non-submerged	Change (%)
Zayande-Rood	239	310	0.297	180	205	0.138
829	225	276	0.226	181	203	0.121
216	208	237	0.139	173	193	0.115
Average	224	274	0.223	178	200.3	0.123

Table 5: Total soluble sugar content (mg g^{-1} DW) of blade under submerged and non-submerged conditions

Rice cultivars	Young Leaves			Old Leaves		
	Submerged	Non-submerged	Change (%)	Submerged	Non-submerged	Change (%)
Zayande-Rood	248	268	0.080	187	185	-0.010
829	231	243	0.052	183	180	-0.016
216	222	235	0.058	178	178	0.000
Average	233.6	248.6	0.064	182.6	181	-0.009

Table 6: Dry weights of panicles and shoot seedlings (g per hill) of three rice cultivars under submerged and non-submerged treatments

Rice cultivars	Shoot		Panicle		1000 - grain weight	
	Submerged	Non-submerged	Submerged	Non-submerged	Submerged	Non-submerged
Zayande-Rood	5.75	5.38	1.65	1.88	24.7	24.5
829	5.84	4.87	1.61	1.54	20.9	19.2
216	6.63	5.88	1.83	1.47	25.5	25.1
Average	6.07	5.38	1.7	1.63	23.7	22.93

Table 7: The numbers of tillers and panicles and grains per hill of three rice cultivars under submerged and non-submerged treatments

Rice cultivars	Tillers			Panicle		
	Submerged	Non-submerged	Change (%)	Submerged	Non-submerged	Change (%)
Zayande-Rood	10	9	-0.1	5	6	0.2
829	11	7	-0.36	5	5	0.0
216	17	11	-0.35	8	7	-0.125
Average	12.67	9.99	-0.29	6	6	0.0

Table 8: The numbers of total, filled and unfilled grains per hill of three rice cultivars under submerged and non-submerged treatments

Rice cultivars	Total grain number		Unfilled grains		Filled grains per panicle	
	Submerged	Non-submerged	Submerged	Non-submerged	Submerged	Non-submerged
Zayande-Rood829216	131123149	126111134	302841	192335	10495116	11191107
Average	134.3	123.6	33	25.67	105	103

From the water saving point of view, there was a dramatic reduction (about 50%) under non-submerged compare to submerged treatment. This finding suggests that through the assessment of mentioned irrigation method it would be possible to 2 fold cultivation land by the same amount of irrigation water.

Effect of Drought Stress on Proline: Accumulation of proline as an osmolyte in our studies occurred to varying in all cultivars. The amount of proline in both young and old leaves substantially increased in plants under drought effect (Table 2, 3). There was a significant variation in proline content among all rice cultivars and between different ages ($P<0.001$) and different parts of the leaves ($P<0.01$) with maximum accumulation in young leaf sheath (sheath) of Zayande-Rood cultivar and minimum in old leaf blades (blades) of 829 cultivar. Interactions between the effect of leaf age and leaf part on proline under different treatments was also statistically significant ($P<0.01$). In all cultivars, higher proline content was observed in young leaves than the old ones. Under un-submerged treatment, proline content in sheath increased significantly more than blades, especially in young leaves. Moreover, Zayande-Rood cultivar shows more amounts of proline in both submerged and non-submerged treatments.

Effect of Drought Stress on Total Soluble Sugars: Total soluble sugars content in both sheath and blade of all three cultivars significantly increased under non-submerged condition, however soluble carbohydrate levels in blade was more than sheath under control plants. The data showed that the highest content of these osmotic adjustments was found in sheath under non-submerged treatment ($P<0.01$) (Table 4, 5). Moreover, soluble sugar content in young leaves was more than old ones ($P<0.01$). The average amount of total soluble sugar in sheath and blade was found as 219 and 212 (mg g^{-1}), respectively. However, these amounts were increased under non-submerged treatment, especially in young leaves. Under non-submerged treatment, sheath of young leaves had the highest soluble carbohydrate level ($274 \text{ mg g}^{-1}\text{DW}$) and the lowest value was found in blades of old leaves ($181 \text{ mg g}^{-1}\text{DW}$). To sum up, Zayande-Rood cultivar accumulates higher amounts of soluble sugars among two other cultivars under non-submerged treatment.

Effects of Drought Stress on Yield Components:

Under non-submerged conditions, total and unfilled grain number were declined markedly ($P<0.05$) (Table 8). Moreover, tiller number per plant was reduced ($P<0.05$), especially in 216 and 829 cultivars. Despite other cultivars, in Zayande-Rood cultivar, the number of panicles and also filled grains was increased under non-submerged treatment, this might be the result in which the panicle dry weight was rose under this condition. (Table 7, 8). Simultaneously, the number of total grains and unfilled grains per panicle was reduced in three cultivars. In terms of 1000 - grain weight, mentioned cultivars show a reduction under non-submerged treatment, however the reduction was higher in 829 cultivar (8.1%) (Table 6).

DISCUSSION

Accumulation of Proline and Soluble Carbohydrates:

The increases in the concentration of proline and soluble carbohydrates in three rice cultivars leaves were found to be remarkable during drought stress. These results suggest that the production of these osmotic adjustments is a common response of plants under drought conditions. The role of proline in adaptation and survival of plants had been observed by Watanabe *et al.* [26] and Saruhan *et al.* [27].

Osmotic adjustment through the accumulation of cellular solutes, such as proline, has been suggested as one of the possible means for overcoming osmotic stress caused by the loss of water [13]. Proline is a non-protein amino acid that forms in most tissues subjected to water stress and together with sugar, it is readily metabolized upon recovery from drought [28]. In addition to acting as an osmo-protectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger [29], as a solute that protects macromolecules against denaturation and as a means of reducing acidity in the cell [30]. However, Vendruscolo *et al.* [11] stated that proline might confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than as an osmotic adjustment.

It has been shown that, the concentration of soluble sugars increased under drought stress in three cultivars. The accumulation of sugars in response to drought stress is also quite well documented [12, 26]. A complex essential role of soluble sugars in plant metabolism is well known as products of hydrolytic processes, substrates in biosynthesis processes, energy production but also in a

sugar sensing and signaling systems. Recently it has been claimed that, under drought stress condition, even sugar flux may be a signal for metabolic regulation [30]. Soluble sugars may also function as a typical osmo-protectant, stabilizing cellular membranes and maintaining turgor pressure. The presence of genes functionally associated with other abiotic stresses among the drought-up-regulated genes suggested that different stresses share some common signaling pathways. Gene ontology attributes such as proline and soluble sugar accumulations were highly enriched in the drought-up-regulated genes, suggesting that those metabolic pathways are important in responses to drought stress. Indeed the importance of many of these pathways to drought tolerance has been empirically supported by transgenic experiments [31].

In our study, the increase of proline and soluble sugar content in leaves of Zayande-Rood cultivar was higher than 829 and 216 cultivars. Therefore; it seems that the accumulation rate was correlated with drought tolerance. Other studies show that the free proline content and soluble sugars can be used as drought tolerance indicators for selecting drought resistant genotypes. One criterion for a character to be an index of drought tolerance is having positive significant correlation coefficient with grain yield under drought stress [32]. In this study proline and soluble sugars showed high significant correlation coefficient with grain under non-submerged treatment, therefore they can be considered as drought tolerance indicators [26, 33].

Submerged plants have lower amount of soluble sugars in sheath than blades. In contrast, under non-submerged condition, sheathes show higher levels of soluble sugar and proline. Previous studies revealed that in rice, usually leaf blades accumulate more sucrose than leaf sheaths [34, 35]. Cabuslay *et al.*, [16] explained that the leaf sheath is the organ most exposed to the stress because water is lost more readily from the sheath than elsewhere and consequently, that water is rapidly drawn from the sheath to replace that lost from blade. This argument seems logical because water is being drawn simultaneously from the sheath by the blade (exposed to vapor pressure deficit) during drought stress. Thus, the leaf sheath would most likely be the primary site for an adaptive response such as osmotic adjustment. An alternative explanation can be based on the observation that solutes usually accumulate in regions where growth is more rapid [36, 37]. Osmotic adjustment can therefore be

seen as a strategy to protect the meristematic tissues during water stress. Since in rice and other grasses the meristematic tissue is enclosed in the leaf sheath, it is expected that the leaf sheath would exhibit a relatively higher rate of osmotic adjustment than the leaf blade or the root [38]. Moreover, such an accumulation of sucrose, with more in the leaf sheath, can be attributed to a rice gene (salt gene), which had its highest expression in the leaf sheath in response to salt and drought stress [39].

In terms of leaves age and their ability to accumulate compatible solutes, it can be seen that in three rice cultivars and also in their sheaths and blades, young leaves show higher proline and soluble sugar contents under both treatments. However, their amounts were higher in non-submerged treatment compared to submerged plants. These results revealed that, under non-submerged treatment, young leaves response more intensive than old leaves, through the higher accumulation of compatible solutes. Related studies show that higher proline and soluble sugars content would be observed in young leaves than the old ones [40]. In addition, it has been documented that the accumulation of compatible solutes under drought was dependent on leaf age whereas, under drought stress conditions, higher content of proline and soluble sugars was found in young leaves. These findings suggest a higher ability to adapt to the change in soil water moisture in young leaves than in old leaves [26, 33].

Shoot Dry Weight and the Number of Tillers per Hill:

Different irrigation treatments and rice cultivars interacted significantly for producing shoot dry matter per hill. Shoot dry matter per hill was the maximum at submerged than non-submerged treatment in all three cultivars, but the lowest reduction was occurred in Zayande-Rood cultivar. Decrease shoot dry matter under lower soil moisture might be due to reduction of leaf area and photosynthesis rate [41, 42].

The results also suggest that Zayande-Rood is drought tolerant and is able to retain green leaves longer than two other cultivars under drought conditions. Retention of green leaves in seedlings under drought conditions has been used as a selection criterion for drought resistance [43]. Alternatively, cultivars with green leaf retention may process dehydration-tolerance mechanism, which allow the plants to maintain metabolic activity, despite low leaf water potential, for example, as a result of high osmotic adjustment [44].

The results showed that the number of tillers per hill was decreased with decreased soil moisture level. The reduction of tillers production under lower soil moisture levels might be the fact that under water stress, plants were not able to produce enough assimilates for inhibited photosynthesis. It might be also happened for less amount of water uptake to prepare sufficient food and inhibition of cell division of meristematic tissue. [42].

Effects of Drought Stress on Yield Components:

According to the result, under non-submerged condition, panicle weight and the number of filled grains in 829 and 216 cultivars has been reduced. In contrast, Zayandeh-Rood cultivar, which not only do not show any reduction in panicle weight and the number of grain, but also there are a higher numbers of filled grains than in submerged plants. The results of 1000-grain weights indicated that the reduction in grain size with drought stress imposed on rice plants in all cultivars. Grain size was found the largest at submerged plants versus non-submerged treatment, especially in 829 cultivar. Similar results on 1000-grain weight under water stress had been reported by Venuprasad *et al.*[40] and Castillo *et al.* [45]. Stress during different growth stages might decrease translocation of assimilates to the grains, which lowered grain weight and increased the empty grains. Results showed that 1000-grain weight varied among different cultivars. Under non-submerged treatment, Zayandeh-Rood cultivar produced the largest grain size, however the smallest grains size was found in 829 cultivar. From this result, it can be concluded that moisture stress influenced the size of the grains and the rate of reduction is depend on the type of cultivar. There are some reports indicated that lower soil moisture might inhibit photosynthesis and decrease translocation of assimilates to the grain which lowered grain weight [46, 47].

Moreover, water stress might lead to a considerable increase in secondary rachis branch abortion and resulted in a reduction in spikelets number per panicle [48]. In addition, drought stress could curtail the kernel sink potential by reducing the number of endosperm cells and amyloplasts formed [49, 50]. Therefore, the rate of reducing in grain weight is correlated to the reduction in the capacity of the endosperm to accumulate starch, in terms of both rate and duration [21].

On the other hand, some studies show that there would be significantly higher gain in biomass (dry weight) after stress imposed. This dry weight would be

associated to the cell division and new material synthesis [48, 51]. Increasing the number of filled grains might be due to the contribution of carbohydrates from current photosynthesis which have been more and efficiently would translocated into the grain and thus increased the grain yield [52]. Recently it has been proposed that grain filling is closely linked to the whole-plant senescence process [20, 21]. Usually, water stress at grain filling induces early senescence and shortens the grain filling period but increases remobilization of assimilates from the straw to the grains [22, 23]. The early senescence induced by a moderate water-deficit during grain filling can enhance the remobilization of stored assimilates and accelerate grain filling of rice [21, 52]. Other experiences show that plants could cope with stress condition exhibiting morphological alteration such as root charactersist in which affect grain formation [53].

Naturally in rice, earlier flowering superior spikelets (grains), usually located on apical primary branches, fill fast and produce larger and heavier grains. While later-flowering inferior spikelets (grains), usually located on proximal secondary branches, are either sterile or fill slowly and poorly to produce grains unsuitable for human consumption [21, 54]. The slow grain-filling rate and low grain weight of inferior spikelets have often been attributed to a limitation in carbohydrate supply [21]. So far, the intrinsic factors responsible for variations in grain filling between the superior and inferior spikelets remain elusive.

CONCLUSION

This finding suggested that Zayandeh-Rood cultivar could be considered as more resistance cultivar against drought condition than 829 and 216 cultivars. Based on this study, genetic scientists may take this founding when selecting the drought tolerant cultivars of rice. Moreover, In arid condition which water is limited and dry land farming is necessary, Zayandeh_Rood could be selected as a tolerance cultivar to water deficiency

ACKNOWLEDGMENTS

Special thanks to the University of Isfahan for financial support and also Department of Biology for laboratory space and facilities. Thanks to Mr Abedi in the Ministry of agriculture division of Isfahan for gifted seeds of different cultivars and technical support.

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