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Influence of drought stress on grain composition and cooking attributes of Iranian rice mutants

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ABSTRACT

Grain quality in rice plays a critical role in consumer acceptance. This research aimed to investigate the grain physicochemical and cooking characteristics of 18 Iranian rice genotypes under both normal conditions and 35 days of drought stress. Significant differences were observed in the studied traits specially percentage of total conversion, head rice, and broken rice indicating differences in the extent of grain retention and damage during processing among the genotypes under both normal and drought stress conditions. The drought stress markedly influenced the expression of nine cooking and nutritional properties and resulted in decreased total conversion percentage, head rice percentage, and cooked head rice length, while the percentage of broken rice increased considerably across all studied genotypes. Conversely, the impact of drought stress on the rough rice length/width ratio, head rice length/width ratio, and elongation ratio were negligible. Most drought-tolerant promising mutant lines exhibited superior grain physicochemical and cooking properties under both drought stress and normal conditions compared to four well-known Iranian rice landraces and cultivars. Evaluation of the grain physicochemical and cooking properties suggested that two drought-tolerant promising mutant lines, namely TM-B-7-1 and HM-250-E-1-1, could be suitable for final cultivar registration experiments.

Key words: Cooking and nutritional quality, Drought, Mutant promising lines, Rice (*Oryza sativa*).

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INTRODUCTION

Rice (*Oryza sativa* L.) is a crop of economic importance with nutritional diversity that contributes to poverty alleviation (Larka *et al.,* 2014). Consumed by people worldwide, it forms the dietary basis for more than half of the world population (Fukagawa and Ziska, 2019). The development and evaluation of rice genotypes with excellent cooking quality and desirable nutritional properties could significantly help address human malnutrition (Gregorio, 2002). Rice quality encompasses (1) physical characteristics such as shape, size, whiteness (milling), head rice, and broken rice; (2) chemical characteristics like amylose content, gel consistency, cooked rice's expansion level, water absorption, and cooking time; and (3) sensory aspects of cooked rice, including color, aroma, hardness, stickiness, and consistency (Kordrostami *et al.,* 2021). The cooking properties of rice are determined by amylose content (AC), gel consistency (GC), and gelatinization temperature (GT) (Wang *et al.,* 2017). Rice with intermediate AC is preferred in most rice-producing areas because it is soft without being overly sticky (Hossaina *et al.,* 2009). GT, a physical trait, affects the cooking time, water absorption, and the temperature at which starch irreversibly loses its crystalline structure during cooking. Rice kernels with low or intermediate GT require less cooking time, a trait desired in high-quality rice varieties (Kim *et al.,* 2021). Volume expansion during cooking is another quality parameter that influences the edible volume, which is the final product after cooking (Mahmood *et al.,* 2023). Grain length, breadth, and length/breadth ratio are crucial factors, especially in cooking, and these largely depend on consumer preference (Grima *et al.,* 2016). Modern varieties tend to have a short to medium bold translucent appearance (Biswas *et al.,* 1992). The milling yield of rice is considered the most critical component of quality (Zhang *et al.,* 2020; Kordrostami *et al.,* 2021), and it significantly determines the grain's market value. Additionally, aroma is a key property in rice marketing, especially in Western and Southern Asia (Thangadurai *et al.,* 2020).

Efforts to improve grain quality parameters and microelement contents of popular rice varieties have been limited so far (Pandey *et al.,* 2013). Ramchander and Ushakumari (2015) studied the quality attributes of rice grains in semi-dwarf and early mutants of White Ponni created using gamma radiation and identified mutants with higher kernel length before and after cooking, Length/Breadth ratio before and after cooking, and linear elongation ratio compared to the wild type. Pandey *et al.* (2013) examined 15 different grain quality parameters of twenty-one indigenous and popular rice varieties in West Bengal, India. They revealed that the percentage of protein, volume expansion ratio, kernel breadth before cooking, head rice recovery, and kernel breadth after cooking were the main contributors to the genetic divergence of these varieties. To select elite germplasms, Abacar *et al.* (2016) evaluated 112 mutants derived from the japonica rice cultivar Wuyujing 3. They found that all rice mutants had short grain lengths (≤5.5 mm) and bold shapes (grain length to width ratio=1.10–2.00). Additionally, all the mutants had milled rice yield values above 50%, AC values below 20%, and protein content values below 10%. They selected 25 rice mutant lines that met the major requirements of Jiangsu standard japonica rice, such as a low percentage of chalky grains, low amylose content, and optimal protein content. Yang *et al.* (2019) noted that while there was no significant effect on the appearance and nutritional quality at the flowering stage, except for a significant increase in chalky kernel and chalkiness under drought stress, drought stress greatly influenced rice physiological traits and yield. Mukamuhirwa *et al.* (2019) investigated the impact of simultaneous drought and temperature stress on the growth, grain yield, and quality characteristics of seven rice cultivars from Rwanda grown in climate chambers. They found that plant development and yield were highly affected by drought, while genotype determined the quality characteristics. Given that grain quality in rice plays a crucial role in consumer acceptance and that drought stress, particularly during the grain-filling period, has detrimental effects on rice grain quality, this research evaluated the grain physicochemical and cooking characteristics of new drought-tolerant mutant rice lines.

In recent years, there has been increasing interest in exploring genetic variations and mutant lines that exhibit enhanced tolerance to drought stress. These mutant lines offer potential solutions to mitigate the adverse effects of water scarcity on rice production. However, it is essential to assess the changes in grain composition and cooking attributes induced by drought stress in these mutant lines to determine their suitability for cultivation under water-limited conditions. This study aims to investigate the influence of drought stress on the grain composition and cooking attributes of Iranian rice mutants. By subjecting these mutant lines to controlled drought conditions, we aim to evaluate changes in important grain characteristics such as amylose content, gelatinization temperature, head rice length, percentage of broken rice, and other key cooking attributes. Understanding these changes will provide valuable insights into the response of rice mutants to drought stress and their potential for cultivation in water-limited environments. By unraveling the effects of drought stress on the grain composition and cooking attributes of Iranian rice mutants, this study contributes to our knowledge of the adaptive responses of rice plants to water scarcity. The findings have implications for breeding programs and agricultural practices aimed at developing drought-tolerant rice varieties with improved grain quality and cooking attributes. Such advancements can ultimately contribute to the sustainable production of rice, ensuring food security and resilience in the face of changing climatic conditions. In this paper, we present the results of our comprehensive analysis of grain composition and cooking attributes of Iranian rice mutants under drought stress conditions. The findings shed light on the potential of these mutants to withstand water scarcity and provide insights for future research and breeding efforts in developing climate-resilient rice varieties.

MATERIALS AND METHODS

Plant materials

In this research, we used two high-quality Iranian rice landraces, Tarom Mahalli, and Hashemi, along with two high-yielding Iranian rice cultivars, Khazar and Gilaneh, as commercial and standard cultivars. In addition, we included 14 promising mutants (M6 generation) rice lines, six originating from Tarom Mahalli landrace, seven from Hashemi landrace, and one from Khazar cultivar. We evaluated the physical and physicochemical properties of these genotypes under normal and drought stress conditions in the Food Analysis Laboratory of the Rice Research Institute of Iran, located in Rasht, Iran. Based on the results of our previous experiments (Ebadi *et al.,* 2016), all 14 promising rice lines demonstrated drought tolerance. In contrast, two landraces, Tarom Mahalli, and Hashemi, along with Gilaneh, an improved cultivar, showed drought sensitivity (Ebadi *et al.,* 2016), with Khazar exhibiting high sensitivity to drought stress (Ebadi *et al.,* 2016). The yield of some promising lines, particularly under drought stress conditions, was two to three times greater than that of the parental and commercial Iranian rice landraces and cultivars.

Evaluation of grain physical and physicochemical properties

In our study, we evaluated a range of physical and physicochemical parameters across 18 genotypes. These included rough rice length, width, and length/width ratio, as well as hulling and milling recovery. We also considered the percentages of broken and head rice, along with head rice length, width, and length/width ratio. Other parameters of interest included gelatinization temperature, amylose content, and head rice length after cooking. We also examined the elongation ratio and the aroma of milled rice after cooking, among several other parameters.

Physical analysis

Ten whole rough rice grains were selected randomly in three sets for our measurements. Using a photo enlarger, the length and width of each grain were measured. The final reading of the length and width of rough rice, expressed in millimeters (mm), was calculated as the average of these ten observations.

The rough rice (paddy) was cleaned and dried it to a moisture content of 12 to 14%. We then weighed out 100-gram samples of the cleaned rough rice and dehulled them using a dehusking machine. The brown rice produced was then polished for bran removal using a polishing machine. After hulling, we milled and polished the brown rice in a Kett polisher for a standard time to determine the milling percentage and head rice recovery. The hulling and milling recovery, or the total conversion percentage of any rice sample, was determined following the method described by Cruz and Khush (2000). We separated the broken grains from the whole grains. The percentage of head rice in any given sample was then calculated, following the methods described by (Cruz and Khush 2000; Ravi *et al.,* 2012).

Length and width of 10 whole grains were measured using a photo enlarger, calibrated to enlarge exactly ten times the original size, as per the methodology detailed by Khush *et al.* (1978). The International Rice Research Institute (IRRI) classifies head rice length into four categories: extra-long (>7.50 mm), long (6.61-7.50 mm), medium (5.51-6.60 mm), and short (<5.50 mm). IRRI also categorizes the shape of head rice grains based on the length-to-width (L/W) ratio, with classifications including slender (>3.0), medium (2.1-3.0), bold (1.1-2.0), and round (1 or less) (Khush *et al.,* 1978).

Physicochemical analysis

The gelatinization temperature (GT) of milled rice were estimated based on its alkali spreading value (ASV). For this, 10 ml of 1.7% potassium hydroxide (KOH) solution was spread on a small glass Petri dish, and two sets of six whole milled grains of rice were evenly spaced in the Petri dish. Kernels were arranged to provide adequate space for spreading. The Petri dishes were then covered and left undisturbed for 23 h at room temperature. The degree of spreading using a 7-point scale as follows: 1 (grain not affected), 2 (grain swollen), 3 (grain swollen, collar incomplete and narrow), 4 (grain swollen, collar complete and wide), 5 (grain split or segmented, collar complete and wide), 6 (grain dispersed, merging with collar), and 7 (grain completely dispersed and intermingled) (Little 1958). The scale further categorized GTs as 1-2: high (74.5-80 °C), 3: high intermediate, 4-5: intermediate (70-74 °C), and 6-7: low (<70 °C).

The amylose content (AC) of milled rice was measured by determining the relative absorbance of the starch-iodine color in a solution of 100-mesh rice flour digest, guided by a standard graph as described in previous methodologies (Williams *et al.,* 1958; Perez and Juliano, 1978). Based on their amylose content, the paddy samples of rice varieties were classified into five groups: waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%), and high (>25%) (Cruz and Khush, 2000).

Cooking properties

The volume expansion ratio, or head rice length after cooking was calculated, by measuring the volumes of both raw and cooked rice. To do this, 10 ml of milled rice was cooked in a boiling water bath until the rice grains were completely gelatinized. After cooking, a micro-scale was used to measure the length of 10 whole rice kernels, from which the average kernel length was determined. Then, the kernel elongation ratio was calculated by dividing the average length of the cooked kernel by the average length of the raw (uncooked) rice (Juliano, 1971).

Aroma content and color of cooked rice

Milled rice samples were cooked and then, raw and cooked rice samples were compared together in terms of aroma. Five panelists were asked to classify the samples as either aromatic or non-aromatic by their smell. The color of cooked rice samples was studied in all studied genotypes. Iranian rice genotypes were derived from *Oryza sativa* L. indica. Good quality indices have alkali spreading value >4. They are classified as grade 1 when grain length is 6.6-7.0 mm, L/W ratio >3.0 and AC 17-22% and as grade 2 when grain length is 5.6-6.5 mm, L/W ratio 2.5-3.0 and AC 23-25% or <17% (Juliano and Villarreal, 1993).

Statistical analysis

The R software was used for cluster and correlation analyses. SAS statistical software version 9.1 was used to carry out the analysis of variance and to examine the varietal differences in physical, cooking, pasting, and milling characteristics of the rice genotypes followed by Duncan's test ($p<0.05$) (Rather *et al.,* 2016).

RESULTS

Before studying the effects of drought stress on the physical and physicochemical parameters of the studied genotypes, the properties of these genotypes were evaluated without imposing stress. Under normal conditions, the rough rice lengths among the 18 genotypes varied from 9.1 mm to 11.1 mm. The longest length of 11.1 mm was observed in Hashemi landrace, while the shortest length of 9.1 mm was found in Tarom Mahalli landrace. Khazar cultivar also showed a shorter length of 9.4 mm. Among the mutant lines, HM-250-6-6 showed the highest length of 10.8 mm, while the shortest of 9.5 mm was observed in KM-200-4-2-E at. Rough rice lengths for the majority of genotypes were centered around 10 mm (Table 1). Looking at the width of the rough rice under normal conditions, the values ranged from 2.3 mm to 2.7 mm across the 18 genotypes. The widest rough rice was observed in the TM series of mutants, all at 2.7 mm. The narrowest rough rice was found in HM-250-6-6, HM-250-7-6, KM-200-4-2-E, Khazar, and Tarom Mahalli genotypes, all measuring 2.3 mm. The rest of the genotypes had widths between these two extremes. Examining the ratio of Rough Rice Length to Rough Rice Width among the genotypes, values were found to range from 3.23 to 4.61. Hashemi had the highest ratio of 4.61, followed by HM-250-7-6 with a ratio of 4.59. On the other hand, HM-250-6-6 had the lowest ratio of 3.23. The rest of the genotypes showed ratios between these extremes, indicating variation in the elongation and shape of the rough rice grains.

Analyzing the percentage of total conversion among the genotypes, values ranged from 67.04% to 75.01%. The highest percentage of total conversion was found in HM-250-6-6 genotype with 75.01%, closely followed by HM-250-E-3-2 with 74.98%. On the other hand, KM-200-4-2-E had the lowest percentage of total conversion at 67.04%. The remaining genotypes exhibited percentages of total conversion between these ranges, indicating variations in the conversion of rough rice to final processed rice. By assessing the percentage of head rice among the genotypes, we found that values ranged from 52.44% to 72.09%. The highest percentage of head rice was observed in HM-250-E-3-2 genotype with 72.09%, closely followed by HM-250-6-6 and HM-250-7-6 with 69.42% and 69.08%, respectively. On the other hand, TM-230-VE-8-4-1 had the lowest percentage of head rice with 52.44%.

Table 1. Grain physicochemical and cooking characteristics of studied rice genotypes in normal condition.

Symbol	Genotype	RRL*	RRW	RRLW Ratio	PTC	PHC	PBR	HRL	HRW	HRLW Ratio	AC	GT	HRLC	El Ratio
M1	TM-230-VE-7-5-1	10	2.7	3.76	73.54	53.52	20.02	7	2.06	3.4	21	4.66	11.1	1.58
M2	TM-230-VE-8-4-1	10.2	2.7	3.84	72.39	52.44	19.95	6.86	2.16	3.18	20.7	4	11.5	1.68
M3	TM-250-10-7-1	9.7	2.7	3.64	74.38	63.94	10.44	7	2.1	3.33	21	4	12.06	1.72
M4	TM-B-2-1-E	10.4	2.7	3.90	74.07	59.76	14.31	7.16	2.16	3.31	21.4	5	11.93	1.67
M5	TM-B-7-1	10.2	2.7	3.81	73.85	52.96	20.89	6.83	1.96	3.48	21.3	4.33	11.8	1.73
M6	TM-B-19-2	9.9	2.7	3.67	73.53	65.45	8.08	7.03	2.13	3.3	21.3	4.66	11.4	1.62
M7	HM-250-E-1-1	10.2	2.6	3.91	74.35	56.35	18	7.03	2.13	3.3	18.7	4	11.96	1.70
M8	HM-250-E-3-2	10.5	2.5	4.26	74.98	72.09	2.89	7.2	2	3.6	20.9	4.33	11.83	1.64
M9	HM-250-6-6	10.8	2.3	3.23	75.01	69.42	5.59	7.16	2	3.58	20.7	4.33	11.8	1.65
M10	HM-250-7-6	10.6	2.3	4.59	74.68	69.08	5.6	7.2	1.96	3.67	21.6	3.5	11.9	1.65
M11	HM-250-12-1	10.4	2.5	4.20	73.68	66.7	6.98	7.26	2	3.63	21.4	4	12	1.65
M12	HM-300-3-1	9.7	2.5	3.82	72.34	66.04	6.3	6.7	2.06	3.25	18.6	5	11.66	1.74
M13	HM-300-5-1	10.4	2.6	4.01	72.05	64.76	7.29	6.36	2.06	3.09	18.8	4.83	10.76	1.69
M14	KM-200-4-2-E	9.5	2.3	4.09	67.04	61.97	5.07	6.66	1.96	3.4	21.8	3.5	10	1.50
H	Hashemi	11.1	2.4	4.61	70.38	63.84	6.54	6.83	1.96	3.48	22	4.66	10.13	1.48
Kh	Khazar	9.4	2.3	4.10	73.87	68.93	4.94	7.13	1.96	3.64	21.9	3.33	12.06	1.69
TM	Tarom Mahalli	9.1	2.3	3.97	68.42	61.79	6.63	6.83	2.03	3.36	22.8	4	10.93	1.60
G	Gilaneh	10.2	2.5	4.14	68.62	55.84	12.78	6.33	1.9	3.33	22.5	4.83	10.8	1.71

RRL*: Rough rice length, RRW: Rough rice width, RRLW Ratio: The ratio of rough rice length/rough rice width, PTC: Percentage of total conversion, PHR: Percentage of head rice, PBR: Percentage of broken rice, HRL: Head rice length, HRW: Head rice width, HRLW Ratio: The ratio of head rice length/head rice width, AC: Amylose content, GT: Gelatinization temperature, HRLC: Head rice length after cooking and El Ratio: Elongation ratio.

The remaining genotypes exhibited percentages of head rice within the range of 55.84% to 68.93%, indicating variations in the yield of intact rice kernels after processing. Examining the percentage of broken rice among the genotypes, values were observed to range from 2.89% to 20.89%. The highest percentage of broken rice was found in TM-230-VE-7-5-1 with 20.89%, closely followed by TM-230-VE-8-4-1 with 19.95%. Conversely, the lowest percentage of broken rice was observed in HM-250-E-3-2 genotype with 2.89%. The remaining genotypes exhibited percentages of broken rice within the range of 4.94% to 12.78%, indicating variations in the extent of rice kernel damage during processing.

Analyzing the head rice length among the genotypes, values were found to range from 6.33 mm to 7.26 mm. The highest head rice length was observed in HM-250-12-1 genotype with 7.26 mm, followed by HM-250-E-3-2 and HM-250-6-6 with lengths of 7.2 mm and 7.16 mm, respectively. Conversely, the lowest head rice length was observed in Gilaneh genotype with 6.33 mm. The remaining genotypes exhibited head rice lengths within the range of 6.36 mm to 7.16 mm, indicating variations in the size of intact rice kernels after processing. Regarding the head rice width among the genotypes, values were observed to range from 1.9 mm to 2.16 mm. The widest head rice width was found in TM-230-VE-8-4-1 and TM-B-2-1-E with 2.16 mm, closely followed by the TM-250-10-7-1 genotype with 2.1 mm. Conversely, the narrowest head rice width was observed in Gilaneh genotype with 1.9 mm. The remaining genotypes exhibited head rice widths within the range of 1.96 mm to 2.13 mm, indicating variations in the width of intact rice kernels after processing. Examining the ratio of head rice length to head rice width among the genotypes, we found values ranging from 3.09 to 3.67. The highest ratio was observed in HM-250-7-6 genotype with 3.67, closely followed by HM-250-12-1 with a ratio of 3.63. Conversely, the lowest ratio was found in HM-300-5-1 genotype at 3.09. The remaining genotypes exhibited ratios within the range of 3.18 to 3.6, indicating variations in the elongation and shape of the head rice grains.

Analyzing the amylose content among the genotypes, we found values ranging from 18.6% to 22.8%. The highest amylose content was observed in Tarom Mahalli and Gilaneh at 22.8% and 22.5%, respectively, closely followed by Hashemi and Khazar with amylose contents of 22% and 21.9%, respectively. On the other hand, the lowest amylose content was observed in the HM-300-3-1 genotype at 18.6%. The remaining genotypes exhibited amylose contents within the range of 18.7% to 21.8%, indicating variation in the starch composition of the rice grains. Examining the gelatinization temperature among the genotypes, we found values ranging from 3.33 to 5. The highest gelatinization temperature was observed in Khazar genotype at 3.33, followed by HM-250-7-6 with a temperature of 3.5. On the other hand, the highest gelatinization temperature was found in Gilaneh at 4.83, followed by HM-300-5-1 with a temperature of 4.83. The remaining genotypes exhibited gelatinization temperatures within the range of 4 to 5, indicating variation in the cooking characteristics and starch properties of the rice grains. Analyzing the head rice length after cooking among the genotypes, we found values ranging from 10.0 mm to 12.06 mm. The highest head rice length after cooking was observed in TM-250-10-7-1 and Khazar, both measuring 12.06 mm. Conversely, the lowest head rice length after cooking was found in KM-200-4-2-E genotype at 10.0 mm. The remaining genotypes exhibited head rice lengths after cooking within the range of 10.76 mm to 11.96 mm, indicating variation in the degree of elongation and texture of the cooked rice grains. Examining the elongation ratio among the genotypes, we found values ranging from 1.48 to 1.74. The highest elongation ratio was observed in HM-300-3-1 at 1.74, closely followed by TM-B-7-1 with a ratio of 1.73. Conversely, the lowest elongation ratio was found in Hashemi at 1.48. The remaining genotypes exhibited elongation ratios within the range of 1.5 to 1.71, indicating variation in the extent of rice kernel elongation during cooking.

Under drought stress conditions, the genotypes exhibited variations in rough rice length. The highest rough rice length was observed in HM-250-7-6 genotype at 10.5 mm, closely followed by TM-230-VE-7-5-1 and HM-250-E-1-1 with lengths of 10.4 mm (Table 2). Conversely, the lowest rough rice length was observed in Tarom Mahalli genotype at 9.0 mm. The remaining genotypes exhibited rough rice lengths within the range of 9.1 mm to 10.4 mm, indicating variation in the size of rice grains under drought-stress conditions. Under drought stress conditions, the genotypes exhibited variations in rough rice width. The highest rough rice width was observed in TM-230-VE-7-5-1, TM-B-2-1-E, and HM-250-E-1-1 genotypes at 2.7 mm. Conversely, the lowest rough rice width was observed in KM-200-4-2-E and Khazar genotypes at 2.1 mm. The remaining genotypes exhibited rough rice widths within the range of 2.2 mm to 2.6 mm, indicating variation in the width of rice grains under drought stress conditions. Under drought stress conditions, the genotypes exhibited variations in the ratio of rough rice length to rough rice width.

Table 2. Grain physicochemical and cooking characteristics of studied rice genotypes in drought stress condition.

Symbol	Genotype	RRL*	RRW	RRLW Ratio	PTC	PHC	PBR	HRL	HRW	HRLW Ratio	AC	GT	HRLC	El Ratio
M1	TM-230-VE-7-5-1	10.4	2.7	3.81	62.38	40.61	21.77	7.06	2.13	3.31	22.2	3.16	11.23	1.59
M2	TM-230-VE-8-4-1	10.3	2.6	3.91	71.32	48.29	23.03	6.93	2.2	3.15	22	3.66	11.46	1.65
M3	TM-250-10-7-1	9.8	2.5	3.99	69.98	34.63	35.35	6.8	2.1	3.23	22	4	11.53	1.7
M4	TM-B-2-1-E	9.7	2.7	3.64	71.49	33.89	37.6	6.76	2.2	3.08	21.8	4.16	11.36	1.68
M5	TM-B-7-1	9.9	2.7	3.67	70.53	33.99	36.54	7.1	2.13	3.33	21.5	3.83	11.83	1.67
M6	TM-B-19-2	9.9	2.5	3.97	71.11	26.51	44.6	6.66	2.1	3.17	21.7	4	11.26	1.69
M7	HM-250-E-1-1	10.2	2.7	3.83	60.61	43.38	17.23	6.8	1.96	3.46	19.3	3.66	11.9	1.75
M8	HM-250-E-3-2	10.4	2.5	4.22	69.83	45.26	24.57	7.1	2	3.55	21.2	4.16	11.36	1.6
M9	HM-250-6-6	10.3	2.5	4.18	74.55	32.41	42.14	7.23	2	3.61	21.2	3.5	11.53	1.59
M10	HM-250-7-6	10.5	2.3	4.49	66.46	18.74	47.72	6.8	1.96	3.46	21.8	3.16	11.2	1.65
M11	HM-250-12-1	10.4	2.4	4.40	73.9	42.31	31.59	6.96	1.96	3.53	21.1	3.16	11.43	1.64
M12	HM-300-3-1	9.6	2.5	3.85	65.3	21.42	43.88	6.3	2	3.15	20.3	3.66	11.23	1.78
M13	HM-300-5-1	9.6	2.5	3.78	63.29	10.84	52.45	6.86	2.06	3.33	20.4	3.33	11.63	1.7
M14	KM-200-4-2-E	9.2	2.1	4.31	69.54	27.78	41.76	6.36	1.9	3.34	21.1	3.33	9.86	1.55
H	Hashemi	9.9	2.3	4.31	70.06	24.15	25.91	6.5	1.93	3.36	21.6	3.33	10.1	1.55
Kh	Khazar	9.1	2.1	4.36	74.1	37.21	36.89	6.86	1.86	3.68	20.8	3.66	11.26	1.64
TM	Tarom Mahalli	9.0	2.2	4.08	65.87	24.69	41.18	6.5	1.83	3.55	23.2	4.16	11.43	1.76
G	Gilaneh	10.3	2.2	4.78	69.88	35.53	34.35	6.43	1.93	3.33	22.7	4.33	11.46	1.78

RRL*: Rough rice length, RRW: Rough rice width, RRLW Ratio: The ratio of rough rice length/rough rice width, PTC: Percentage of total conversion, PHR: Percentage of head rice, PBR: Percentage of broken rice, HRL: Head rice length, HRW: Head rice width, HRLW Ratio: The ratio of head rice length/head rice width, AC: Amylose content, GT: Gelatinization temperature, HRLC: Head rice length after cooking El Ratio: Elongation ratio.

The highest ratio was observed in Gilaneh genotype at 4.78, indicating a relatively longer length compared to the width of the rice grains. Conversely, the lowest ratio was observed in Tarom Mahalli genotype at 4.08. The remaining genotypes exhibited ratios within the range of 3.64 to 4.49, indicating variations in the elongation and shape of rice grains under drought-stress conditions.

Under drought stress conditions, the genotypes exhibited variations in the percentage of total conversion. The highest percentage of total conversion was observed in HM-250-6-6 genotype at 74.55%, indicating a higher proportion of converted rice grains. Conversely, the lowest percentage of total conversion was observed in HM-250-E-1-1 genotype at 60.61%. The remaining genotypes exhibited percentages of total conversion within the range of 63.29% to 74.10%, indicating variations in the degree of conversion of rice grains under drought-stress conditions. Under drought stress conditions, the genotypes exhibited variations in the percentage of head rice. The highest percentage of head rice was observed in TM-230-VE-8-4-1 genotype at 48.29%, indicating a higher proportion of intact rice grains. Conversely, the lowest percentage of head rice was observed in HM-300-5-1 genotype at 10.84%. The remaining genotypes exhibited percentages of head rice within the range of 18.74% to 45.26%, indicating variations in the extent of grain retention after processing under drought-stress conditions. Under drought stress conditions, the genotypes exhibited variations in the percentage of broken rice. The highest percentage of broken rice was observed in HM-300-5-1 genotype at 52.45%, indicating a higher proportion of damaged or fragmented rice grains. Conversely, the lowest percentage of broken rice was observed in HM-250-E-1-1 genotype at 17.23%. The remaining genotypes exhibited percentages of broken rice within the range of 23.03% to 47.72%, indicating variations in the extent of grain breakage or damage during processing under drought-stress conditions.

The head rice length of the genotypes under drought stress conditions varied. The highest head rice length was observed in HM-250-7-6 genotype with 7.23 mm, indicating longer rice grains. Conversely, the lowest head rice length was observed in HM-300-3-1 genotype with 6.3 mm. The remaining genotypes exhibited head rice lengths within the range of 6.36 mm to 7.1 mm, indicating variations in the length of intact rice grains after processing under drought-stress conditions. The head rice width of the genotypes under drought stress conditions showed variations. The widest head rice width was observed in TM-230-VE-8-4-1 genotype at 2.2 mm. On the other hand, the narrowest head rice width was observed in Tarom Mahalli and Gilaneh genotypes with 1.83 mm and 1.93 mm, respectively. The remaining genotypes exhibited head rice widths within the range of 1.9 mm to 2.13 mm, indicating variations in the width of intact rice grains after processing under drought-stress conditions. The ratio of head rice length to head rice width varied among the genotypes under drought stress conditions. The highest ratio was observed in Khazar (3.68), indicating a relatively longer length compared to its width. On the other hand, TM-B-2-1-E genotype had the lowest ratio at 3.08, indicating a relatively shorter length compared to its width. The remaining genotypes exhibited ratios within the range of 3.15 to 3.61, indicating variations in the elongation of rice grains after processing under drought-stress conditions.

The amylose content of the genotypes under drought stress conditions varied. Tarom Mahalli exhibited the highest amylose content with 23.2%, indicating a higher proportion of amylose in the rice grains. Gilaneh also had a relatively high amylose content with 22.7%. On the other hand, HM-300-3-1 and HM-300-5-1 genotypes had the lowest amylose content with 20.3% and 20.4%, respectively. The remaining genotypes showed amylose content within the range of 20.8% to 22.2%, indicating variations in the starch composition of the rice grains under drought-stress conditions. The gelatinization temperature of the genotypes under drought stress conditions varied. The genotypes exhibited a range of gelatinization temperatures from 3.16 °C to 4.33 °C. Gilaneh had the highest gelatinization temperature of 4.33 °C, indicating a higher temperature required for the starch in the rice grains to gelatinize. Tarom Mahalli and TM-B-2-1-E genotypes also showed relatively high gelatinization temperatures of 4.16 °C. On the other hand, HM-250-7-6, HM-250-12-1, HM-300-5-1, KM-200-4-2-E, Hashemi, Khazar, and TM-230-VE-8-4-1 genotypes exhibited a gelatinization temperature of 3.33 °C, indicating a lower temperature required for gelatinization. The remaining genotypes exhibited gelatinization temperatures within the range of 3.16 °C to 3.66 °C, indicating variations in the gelatinization properties of the rice grains under drought stress conditions.

The head rice length after cooking varied among the different genotypes under drought stress conditions. The genotypes exhibited head rice lengths ranging from 9.86 mm to 11.83 mm. The genotype TM-B-7-1 had the highest head rice length after cooking at 11.83 mm, followed closely by HM-250-E-1-1, HM-300-

5-1, and Gilaneh with head rice lengths of 11.9 mm, 11.63 mm, and 11.46 mm, respectively. On the other hand, KM-200-4-2-E had the lowest head rice length after cooking at 9.86 mm. The remaining genotypes exhibited head rice lengths after cooking within the range of 10.1 mm to 11.53 mm, indicating variations in the extent of elongation of the rice grains during the cooking process under drought-stress conditions. The elongation ratio, which represents the extent of elongation of rice grains during the cooking process, varied among the different genotypes under drought stress conditions. The genotypes exhibited elongation ratios ranging from 1.55 to 1.78. HM-300-3-1 and Gilaneh had the highest elongation ratios at 1.78, indicating significant elongation of the rice grains upon cooking. Similarly, Tarom Mahalli and HM-250-E-1-1 showed relatively high elongation ratios of 1.76 and 1.75, respectively. On the other hand, KM-200-4-2-E and Hashemi had the lowest elongation ratios of 1.55, indicating minimal elongation during cooking. The remaining genotypes exhibited elongation ratios within the range of 1.59 to 1.7, indicating moderate elongation of the rice grains. These variations in elongation ratio reflect the differences in the cooking characteristics of the genotypes under drought stress conditions.

Interactions of genotype×environment on grain physicochemical and cooking characteristics were investigated in a three-replicated randomized complete block design, and the obtained data were analyzed using SAS software. Results indicated that there were no significant differences among the studied genotypes regarding: rough rice length/width ratio, head rice length/width ratio, and elongation ratio. In other words, the impact of drought stress on these traits was not considerable. However, the effect of drought stress on the expression of traits such as rough rice length and width, percentage of head rice, percentage of broken rice, head rice length, gelatinization temperature, and head rice length after cooking was significant ($p=0.01$). Additionally, the effect of the environment on the percentage of total conversion and amylose content was significant at the 5% level. The 35 days of drought stress had a tremendous impact on the expression of nine cooking and nutritional properties of the studied genotypes (Table 3).

In addition, there were no significant intra-genotypic differences at the 1% and 5% levels for the percentage of broken rice. However, when drought stress was imposed on all studied genotypes, the percentage of total conversion, percentage of head rice, and head rice length after cooking significantly decreased, while the percentage of broken rice considerably increased

Table 3. Statistical analysis of grain physiochemical and cooking characteristics of studied rice genotypes in two normal and drought stress conditions.

Source of variation	df	Mean of square												
		RRL*	RRW	RRLWR	PTC	PHR	PBR	HRL	HRW	HRLWR	AC	GT	HRLC	El Ratio
Environment	1	1.1614**	0.1134**	0.0027^{ns}	389.0065*	24675.2375**	18886.8871**	0.5647**	0.0118*	0.0306^{ns}	4.7292*	9.6421**	1.1886**	0.0004^{ns}
Treatment	17	1.1845**	0.1704**	0.4620**	37.1805**	145.0093*	93.8611^{ns}	0.3406**	0.0448**	0.1518**	6.0292*	0.7966**	1.7512**	0.0306**
Error	89	0.0968	0.0081	0.0434	7.4702	52.3388	57.3498	0.0223	0.0025	0.0068	0.1769	0.0895	0.0579	0.0017
Coefficient of variation (%)		3.1318	3.6407	5.1080	3.8655	15.2304	32.4022	2.1835	2.4733	2.4359	1.9821	7.5223	2.1143	2.5077

RRL*: Rough rice length, RRW: Rough rice width, RRLWR: Rough rice length/width ratio, PTC: Percentage of total conversion, PHR: Percentage of head rice, PBR: Percentage of broken rice, HRL: Head rice length, HRW: Head rice width, HRLWR: Head rice length/width ratio, AC: Amylose content, GT: Gelatinization temperature, GLC: Grain length after cooking and El Ratio: Elongation ratio.

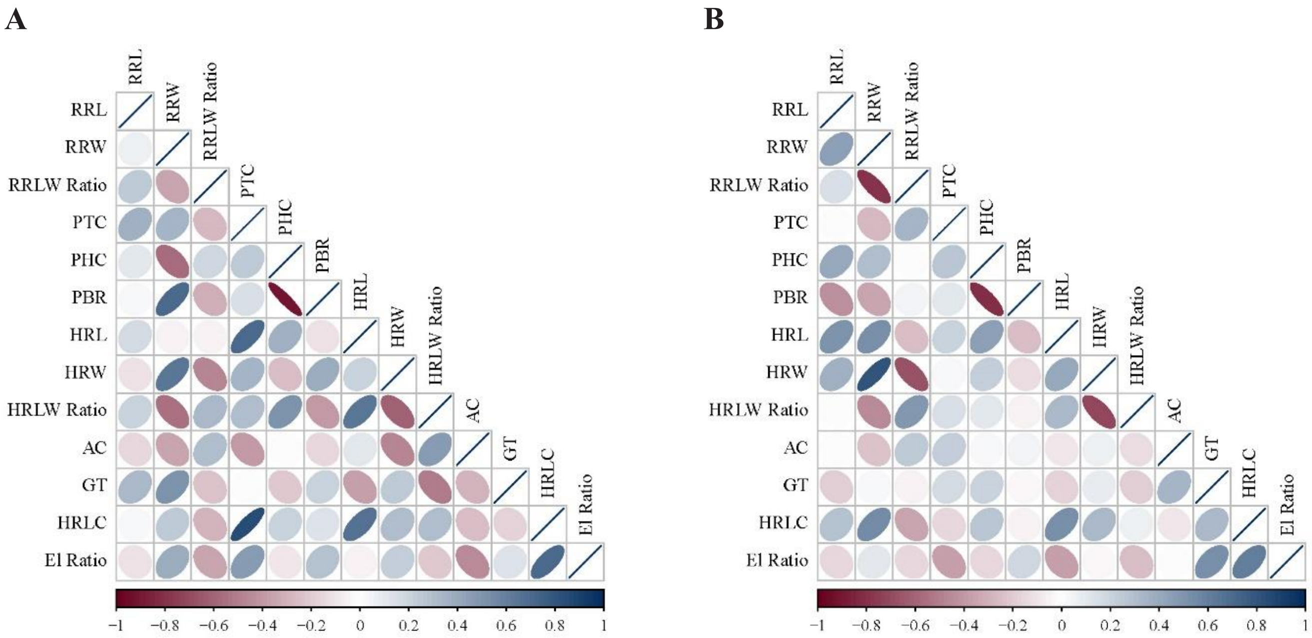

Figure 1. Correlation analysis of the studied traits under **A:** normal and **B:** drought stress conditions.
RRL: Rough rice length, RRW: Rough rice width, RRLWR: Rough rice length/width ratio, PTC: Percentage of total conversion, PHR: Percentage of head rice, PBR: Percentage of broken rice, HRL: Head rice length, HRW: Head rice width, HRLWR: Head rice length/width ratio, AC: Amylose content, GT: Gelatinization temperature, GLC: Grain length after cooking and El Ratio: Elongation ratio.

(Table 3). The correlation analysis results under normal conditions revealed positive correlations between the percentage of broken rice and rough rice width, head rice length and percentage of total conversion, head rice width and rough rice width, head rice length/head rice width ratio and head rice length, head rice length after cooking and percentage of total conversion, head rice length after cooking and head rice length, and elongation ratio and head rice length after cooking (Figure 1).

Furthermore, a negative correlation was observed between the percentage of broken rice and the percentage of head rice, as well as between the percentage of head rice and rough rice width. Under drought stress conditions, positive correlations were found between rough rice width and head rice width, head rice length after cooking and rough rice width, head rice length after cooking and head rice length, elongation ratio, and head rice length after cooking. Additionally, a negative correlation was identified between the ratio of rough rice length/rough rice width and rough rice width.

The results of the cluster analysis are presented in Figure 2. Under normal conditions, the genotypes were clustered into three groups. The first cluster included HM-300-3-1, HM-300-5-1, TM-B-2-1-E, TM-250-10-7-1, TM-B-19-2, TM-230-VE-8-4-1, HM-250-E-1-1, TM-230-VE-7-5-1, and TM-B-7-1 genotypes. These genotypes exhibited superior performance in traits such as gelatinization temperature (GT), elongation ratio (El Ratio), head rice width (HRW), rough rice width (RRW), and percentage of broken rice (PBR). They also had the minimum values for amylose content (AC), head rice length (HRL), percentage of total conversion (PTC), head rice length after cooking (HRLC), and the ratio of head rice length to width (HRLW ratio). In the second cluster, HM-250-6-6, HM-250-E-3-2, HM-250-7-6, HM-250-12-1, and Khazar cultivars were grouped. These genotypes demonstrated high values for AC, HRL, PTC, HRLC, and HRLW ratio traits. In the last cluster, Tarom Mahalli, Hashemi, and Gilaneh cultivars were clustered together. These genotypes were distinct from the others under normal conditions.

Under drought stress conditions, HM-250-E-1-1, TM-B-7-1, TM-B-2-1-E, TM-250-10-7-1, TM-B-19-2, TM-230-VE-7-5-1, and TM-230-VE-8-4-1 genotypes were clustered together. These genotypes exhibited superior performance in RRW, HRW, percentage of head rice (PHC), rough rice length (RRL), HRL, HRLC, and El Ratio. In the second cluster, the other genotypes were grouped (Figure 2).

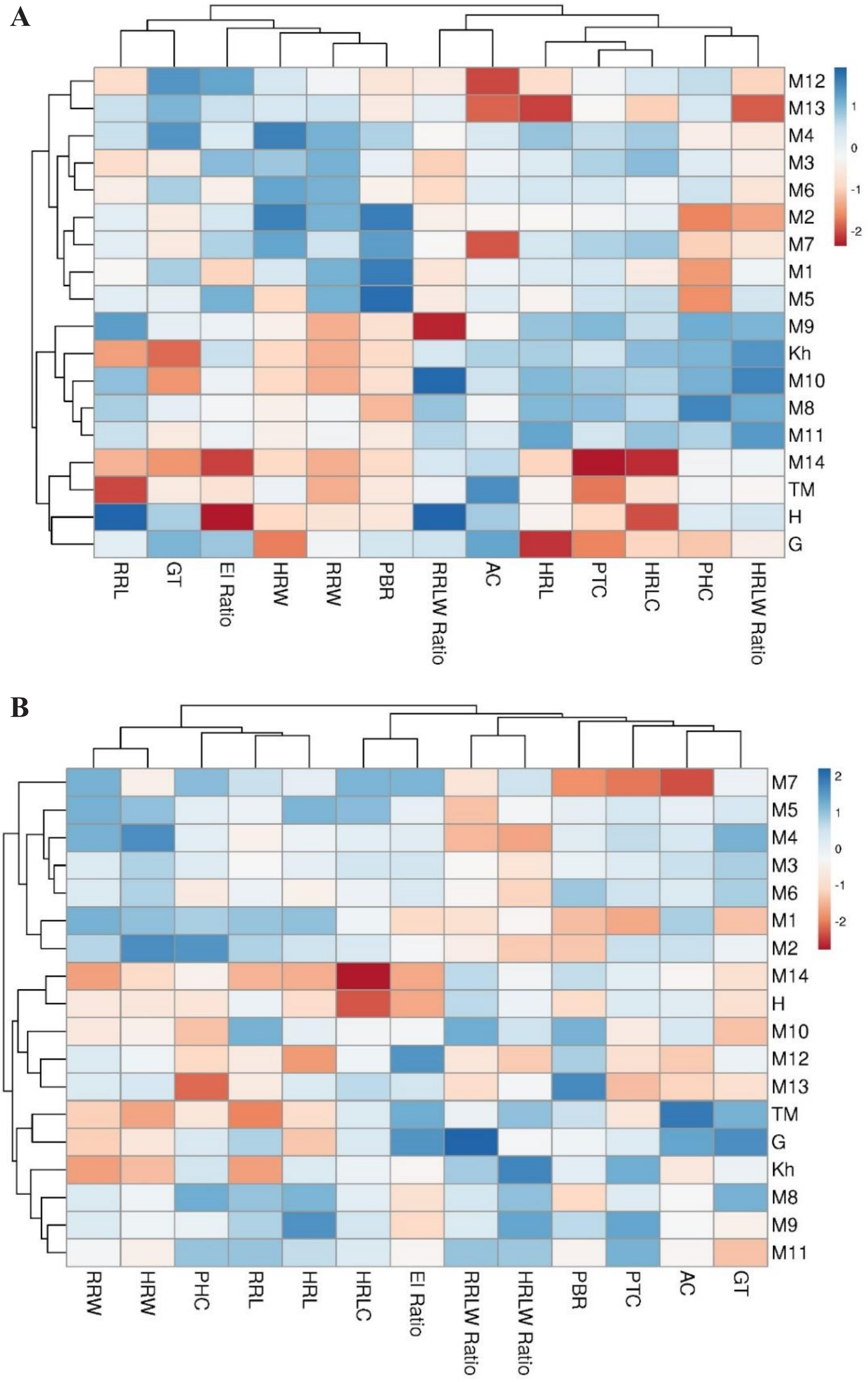

Figure 2. Cluster analysis of the studied genotypes under **A:** normal and **B:** drought stress conditions.
RRL: Rough rice length, RRW: Rough rice width, RRLWR: Rough rice length/width ratio, PTC: Percentage of total conversion, PHR: Percentage of head rice, PBR: Percentage of broken rice, HRL: Head rice length, HRW: Head rice width, HRLWR: Head rice length/width ratio, AC: Amylose content, GT: Gelatinization temperature, GLC: Grain length after cooking and El Ratio: Elongation ratio.

DISCUSSION

Mukamuhirwa *et al.* (2019) conducted a study on rice genotypes and found that plant development and yield were greatly affected by drought, while the impact on grain quality characteristics varied among the genotypes. This aligns with our findings, as we observed significant changes in grain quality under drought stress conditions. The research by Dingkuhn and Gal (1996) also supports the influence of soil water deficiency, particularly during the grain-filling period, on rice grain quality.

Interestingly, the results of Renmin and Yuanshu (1989) differed from our findings. They reported an increase in milled rice recovery and brown rice protein content, along with a decrease in the percentage of unripened grain and amylose content when soil moisture content decreased. In contrast, our research showed a decrease in the percentage of total conversion under drought stress, accompanied by an increase in amylose content for most studied genotypes. These disparities might be attributed to variations in rice genotypes, experimental conditions, or other factors.

Furthermore, Pandey *et al.* (2014) observed an increase in the head-rice ratio under moisture stress conditions, suggesting that drought during the grain ripening stage could reduce broken grains and result in a higher proportion of intact head rice. However, our research demonstrated a negative impact of drought on the percentage of head rice, leading to a decrease in head rice and an increase in the percentage of broken rice. These discrepancies could be due to different experimental setups, rice varieties, or specific drought conditions.

It is worth noting that a positive correlation between gelatinization temperature and cooking time has been established (Veronic *et al.,* 2007). Therefore, the occurrence of moisture stress during the ripening stage could potentially shorten the cooking time of rice grains.

In summary, while there are similarities between our findings and previous research, there are also discrepancies that could be attributed to variations in rice genotypes, experimental conditions, and the specific impact of drought stress on grain quality characteristics. Further studies are warranted to explore these differences and gain a deeper understanding of the complex relationship between drought stress and rice grain quality.

In our research, we observed a decrease in the gelatinization temperature of most studied genotypes under drought stress, except for three genotypes. This differs from the findings of Pandey *et al.* (2014), who reported that moisture stress during the ripening stage led to higher grain volume expansion upon cooking, as indicated by increased peak viscosity time and "breakdown" values. In contrast, our results showed a decrease in head rice length and grain volume expansion upon cooking for the majority of studied genotypes under drought stress conditions.

Additionally, Pandey *et al.* (2014) found that amylose content generally decreased under water stress conditions. They observed lower gelatinization temperature and lower peak viscosity in stressed rice grains. However, in our research, the amylose content of most studied genotypes increased under drought stress, and they exhibited higher gelatinization temperatures. These disparities highlight the complex nature of the response of rice grain properties to drought stress, which can vary depending on the specific genotypes and experimental conditions.

Among the 18 genotypes evaluated in our research, two drought-tolerant promising lines, TM-B-7-1 and HM-250-E-1-1, stood out as superior genotypes in terms of grain physicochemical and cooking properties. These genotypes exhibited unique characteristics compared to the four Iranian famous rice landraces and cultivars. Based on these findings, TM-B-7-1 and HM-250-E-1-1 have the potential to be recommended for further evaluation and potential registration as cultivars in future experiments.

Overall, our research highlights the variability in grain physicochemical and cooking properties among different genotypes under drought stress. The identification of promising drought-tolerant lines opens up possibilities for developing rice varieties that can withstand water scarcity while maintaining desirable grain quality characteristics.

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