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TRITICALE (× *TRITICOSECALE* WITTMACK) EVALUATION FOR DROUGHT STRESS TOLERANCE BASED ON MORPHO-PHYSIOLOGICAL TRAITS AND DNA MARKERS

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SUMMARY

This latest study aimed to evaluate the triticale cultivars for drought tolerance through morphophysiological traits and molecular analyses. Under artificial drought conditions induced by the polyethylene glycol (PEG-6000), the triticale cultivars Sergiy, Xlebarob, and K. Prag exhibited the highest tolerance and demonstrated better adaptability to drought stress conditions. Molecular analysis identified the alleles (Xgwm484, Xmc525, and Xgwmc389) associated with drought tolerance based on DNA markers, establishing these markers as reliable indicators for selection. Specifically, alleles with 255 bp (Xgwm484), 230 bp (Xmc525), and 260 bp (Xgwmc389) were indicative of drought tolerance, confirming the highest drought tolerance in the cultivars K. Prag, Sergiy, and Xlebarob. These cultivars showed potential for future breeding using molecular techniques like gene pyramiding to enhance drought resistance and are suitable as elite initial material for the selection process. The phylogenetic analysis revealed genetic similarities and differences among the triticale cultivars, highlighting the crucial role of drought tolerance-related markers in selection and ensuring sustainable productivity. The results provided fundamental insights for determining the drought tolerance levels among triticale cultivars and defining future selection directions.

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Key findings: The study identified drought-tolerant alleles in triticale cultivars using markers Xgwm484, Xmc525, and Xgwmc389. The allele Xgwm484 with 255 bp appeared in cultivars Sardor, K. Prag, Sergiy, and Xlebarob, while the allele Xmc525 with 230 bp was evident in triticale cultivars Tixon, Kunak, Yarillo, K. Prag, Sergiy, Xlebarob, and Prao-02, confirming their highest drought tolerance.

INTRODUCTION

Recently, global climate change has considerably affected crop production, with water scarcity and drought emerging as major stressors for cereal crops, including Triticale (× *Triticosecale* Wittmack), posing critical challenges to cultivation. Triticale is an artificial hybrid of wheat and rye, distinguishable for its high yield, nutritional values, and environmental resilience. However, certain cultivars exhibited sensitivity to ecological stressors, particularly under drought stress conditions. Consequently, identifying drought-tolerant Triticale genotypes and developing resilient cultivars have become the main focus of modern breeding, physiological, and genetic research (Bano and Singh*,* 2019; Suvi *et al.,* 2021; Yuldashov *et al.,* 2023).

Drought stress severely affects seed germination, seedling growth and development, and ultimately, crop yield. In triticale, drought represents a primary ecological constraint limiting seed germination and seedling growth (Liu *et al.,* 2021; Matniyazova *et al.,* 2024). Therefore, the identification and development of drought-tolerant field-grown triticale genotypes through effective breeding methods is of paramount importance (Baboev *et al.,* 2020; Meliev *et al.,* 2023). Drought, a challenging global concern, also considerably alters the triticale. In Uzbekistan, triticale cultivation primarily serves as a secondary crop (Buronov *et al.,* 2023; Bakhadirov *et al.,* 2024). Being a wheat and rye hybrid, triticale demonstrates greater resistance to various diseases, with higher yield and increased fodder production than wheat.

For this reason, the ensuing research focused on investigating triticale's drought tolerance under laboratory conditions, utilizing sucrose and polyethylene glycol (PEG-6000) to assess plant responses to artificially developed drought stress conditions. Currently, PEG-6000 is a widely used treatment to evaluate plant drought tolerance. PEG-6000 application typically simulates the artificial water deficiency and model drought stress in plants (Eff). The use of PEG commonly helps detect drought stress in rice plants (Lafitte *et al.,* 2007). It is well-known that PEG does not penetrate the cell wall apoplast, and due to its high molecular weight (above 3000 Da), no absorption of PEG molecules occurs (Verslues *et al.,* 2006). Polyethylene glycol serves to induce drought stress in crop plants (Dodig *et al.,* 2010; Omonov *et al.,* 2023).

High PEG concentrations (15%–25%) considerably reduce the root number and length. For instance, Muscolo *et al.* (2014) evaluated triticale genotypes in a 20% PEG solution and found a decrease in root length by 35%–45%, a phenomenon linked to restricted water uptake due to osmotic stress. Triticale-tolerant genotypes maintain stable root numbers at 10% PEG (Grzesiak *et al.,* 2019). These indicators contribute to more understanding of rice plant adaptation mechanisms to drought stress conditions (Parvez, 2021). Notably, identifying early phenotypic responses is essential for expediting the selection process.

However, relying solely on phenotypic traits for evaluation is often insufficient, as these traits may not fully reflect genotypic stability under varied environmental conditions. Therefore, genetic analysis, particularly the use of molecular markers, enables the determination of drought tolerance at the genetic level, enhancing selection efficiency (Makhmudov *et al.,* 2025). The use of DNA markers, especially simple sequence repeat (SSR) markers, facilitates the analysis of genetic variations among genotypes, allelic diversity, and the presence of stress-related genes (Bano and Singh, 2019). This approach enables the integration of molecular breeding techniques, allowing for rapid and precise identification of crop-tolerant genotypes.

Combining morphophysiological and molecular approaches is considerably one of the most effective strategies in triticale breeding. Identifying drought-tolerant cultivars stabilizes yields and supports the development of sustainable agriculture. This is particularly viable and feasible for countries like Uzbekistan, which have arid and semi-arid regions, where enhanced drought tolerance of triticale cultivars remains a strategic priority. From this perspective, the presented study aimed to investigate the morphophysiological responses of triticale cultivars to drought stress induced by varying concentrations of PEG-6000 and assess their tolerance levels using DNA markers. This approach facilitates the identification of the triticale-tolerant genotypes for effective utilization in future selection and breeding programs.

MATERIALS AND METHODS

Plant material and procedure

This study utilized systematic samples of the 10 Triticale (× *Triticosecale* Wittmack) cultivars (Table 1). Their drought tolerance assessment and molecular analysis sought to identify associated DNA markers. All the laboratory work transpired at the Scientific Research Institute of Plant Genetic Resources, Ministry of Agriculture, Uzbekistan. In the concerned study, the drought tolerance of 10 triticale cultivars entailed determination under various concentrations of PEG-6000 (5%, 10%, 15%, 20%, and 25%), by following the methodology of Michel and Kaufmann (1973).

The drought tolerance evaluation of the triticale cultivar samples followed the methodology of Raja *et al*. (2017). Triticale seeds' growing for seven days occurred in distilled water and in PEG-6000 solutions at concentrations of 5%, 10%, 15%, 20%, and 25%. The drought tolerance index (DTI) and damage index (DI) attained calculations for the cultivar samples. The DTI reflects the plant's tolerance to drought, while the DI indicates the degree of damage caused by drought stress conditions, with the formula as follows.

$$DTI = 1 - \frac{\text{Average SL in distilled water} - \text{Average SL in 5\% PEG}}{\text{Average SL in distilled water}}$$

$$DI = \frac{\text{Average SL in distilled water} - \text{Average SL in 5\% PEG}}{\text{Average SL in distilled water}}$$

Where SL represents the shoot length (cm).

Table 1. Triticale cultivars and their origins used in the study.

No.	Cultivar Name	Origin
1	Do‘stlik-4	Uzbekistan
2	Tixon	Russia
3	Kunak	Russia
4	Yarillo	Russia
5	Sardor	Uzbekistan
6	K.Prag	Uzbekistan
7	Sergiy	Russia
8	Xlebarob	Russia
9	PRAO-02	Russia
10	PRAG-48/1	Russia

Table 2. Panel of DNA markers genetically associated with drought tolerance.

No.	Marker name	PrimerSequence5′-3′	Chr.	References
1	Xgwm626	GATCTAAAATGTTATTTTCTCTC TGACTATCAGCTAAACGTGT	6B	Roder *et al*. (1998), Dodig *et al*. (2010)
2	Xpsp3200	GTTCTGAAGACATTACGGATG GAGAATAGCTGGTTTTGTGG	6D	Sunilkumar *et al*. (2023)
3	Xgwm337	CCTCTTCCTCCCTCACTTAGC TGCTAACTGGCCTTTGCC	1D	Dodig *et al.* (2010)
4	Xwmc118	AGAATTAGCCCTTGAGTTGGTC CTCCCATCGCTAAAGATGGTAT	5B	Somers *et al*. (2004)
5	Xgwm484	ACATCGCTCTTCACAAACCC AGTTCCGGTCATGGCTAGG	2D	Sunilkumar *et al*. (2023)
6	Wmc488	AAAGCACAACCAGTTATGCCAC GAACCATAGTCACATATCACGAGG	2B	Sunilkumar *et al*. (2023)
7	Wmc525	GTTTGACGTGTTTGCTGCTTAC CTACGGATAATGATTGCTGGCT	2B	Sunilkumar *et al*. (2023)
8	Xgwm389	ATCATGTCGATCTCCTTGACG TGCCATGCACATTAGCAGAT	3B	Roder *et al.* (1998), Dodig *et al.* (2010),
9	Xwmc78	AGTAAATCCTCCCTTCGGCTTC AGCTTCTTTGCTAGTCCGTTGC	3B	Somers *et al.* (2004)

DNA extraction

The genomic DNA extraction came from young leaves of freshly harvested triticale plants using a slightly modified CTAB (cetyltrimethylammonium bromide) method using liquid nitrogen. The quality and quantity of the DNA samples, as determined, used a NanoDrop Eight spectrophotometer (Thermo Fisher Scientific, USA). A panel of DNA markers associated with drought tolerance in the triticale genome underwent investigation and selection (Table 2).

SSR analysis and gel-electrophoresis

Polymerase chain reaction (PCR)-based SSR genotyping proceeded according to the method described in previous studies (Bakhadirov *et al*., 2024). Nine SSR markers associated with drought tolerance obtained selection from the database (Table 2). The "hot-start" PCR program used consisted of 35 cycles, with a denaturation step at 94 °C for 20 s, an annealing temperature ranging from 55 °C to 60 °C for 30 s, and an elongation step at 72 °C for 50 s.

The conduct of gel electrophoresis on a 2.5% agarose gel helped determine the molecular weight of the PCR products. The agarose gel incurred staining with ethidium bromide before visualization using the GelDoc Go Gel Imaging System (Bio-Rad, USA). The AmpliSize Molecular Ruler (Bio-Rad, USA) succeeded in measuring amplicon sizes. Phylogenetic tree construction and visualization continued using the MEGA 11 software.

RESULTS AND DISCUSSION

Based on laboratory analysis results, triticale cultivars grown in solutions with different PEG-6000 concentrations (5%, 10%, 15%, 20%, and 25%) progressed. After seven days of the experiment, the adaptability, root length, and shoot length of the plants involved their measurement. The results indicated nearly all cultivars exhibited 100% adaptability in 5% and 10% PEG-6000 solutions. As the PEG solution concentration enhanced, the adaptability decreased gradually. Despite the influence of 25% PEG, the triticale cultivars K. Prag, Xlebarob, and Sergiy maintained the highest adaptability at 96.7%, while genotypes Sardor, Yarillo, and Tixon were at 85.0%, and the triticale cultivars PRAO-02, PRAG-48/1, Do`stlik-4, and Kunak at 80.0% (Table 3). Ashraf *et al.* (2018) reported that plants showed the highest adaptability under low osmotic stress; however, this adaptability decreases as PEG concentrations increase.

Table 3. Germination rates of triticale cultivars in PEG-6000 solutions.

No.	Cultivars	Control Germination	5% Germination	10% Germination	15% Germination	20% Germination	25% Germination
1	Do'stlik-4	100%	100%	90%	90.00%	85.00%	80.00%
2	Tixon	100%	100%	93.33%	93.33%	90.00%	85.00%
3	Kunak	100%	100%	90%	90.00%	85.00%	80.00%
4	Yarillo	100%	100%	93.33%	93.33%	90.00%	85.00%
5	Sardor	100%	100%	100%	93.33%	90.00%	85.00%
6	K.Prag	100%	100%	100%	100%	96.70%	96.70%
7	Sergiy	100%	100%	100%	100%	96.70%	96.70%
8	Xlebarob	100%	100%	100%	100%	96.70%	96.70%
9	PRAO-02	100%	100%	93.33%	90.00%	85.00%	80.00%
10	PRAG-48/1	100%	100%	90%	90.00%	85.00%	80.00%

Table 4. Drought tolerance index (DTI) and damage index (DI) of triticale cultivars.

No.	Cultivars	DI-5%	DTI-5%	DI-10%	DTI-10%	DI-15%	DTI-15%	DI-20%	DTI-20%	DI-25%	DTI-25%
1	Do'stlik-4	27.80	72.20	41.04	58.96	50.24	49.76	75.46	24.54	95.97	4.03
2	Tixon	22.81	77.19	37.26	62.74	43.02	56.98	69.84	30.16	91.48	8.52
3	Kunak	33.53	66.47	43.67	56.33	51.77	48.23	75.17	24.83	96.37	3.63
4	Yarillo	17.72	82.28	36.27	63.73	41.80	58.20	67.44	32.56	91.01	8.99
5	Sardor	10.57	89.42	33.71	66.29	40.65	59.35	66.89	33.11	90.42	9.58
6	K.Prag	8.78	91.22	30.56	69.44	38.28	61.72	65.15	34.85	89.77	10.23
7	Sergiy	1.15	98.85	24.65	75.35	27.21	72.79	49.88	50.12	83.18	16.82
8	Xlebarob	3.16	96.84	27.99	72.01	27.99	65.07	54.82	45.18	84.12	15.88
9	PRAO-02	24.57	75.43	39.93	60.07	44.21	55.79	70.21	29.79	94.64	5.36
10	PRAG-48/1	35.90	64.10	46.02	53.98	53.98	46.02	81.74	18.26	97.05	2.95

They also highlighted osmotic adjustment as a key mechanism for maintaining plant viability in stress conditions.

According to laboratory results, the experiments conducted under simulated drought conditions using 20% and 25% concentrations of PEG-6000 revealed the highest drought tolerance in specific triticale cultivars. Notably, the cultivars Sergiy, Xlebarob, and K. Prag demonstrated significantly higher drought tolerance indices than other cultivars, achieving a germination rate of 96.7%. These elevated tolerance indices indicate these triticale cultivars possess the genetic traits conferring resilience to drought stress conditions. These results align with the research of Majd *et al*. (2019), who emphasized triticale's high germination rates within stress conditions make it a valuable material for breeding purposes. Hussain *et al.'s* (2020) findings disclosed particular wheat cultivars maintained the highest germination under drought stress due to genetic factors. These findings considerably support the optimum drought tolerance observed in triticale cultivars Sergiy, Xlebarob, and K. Prag, highlighting their potential for breeding resilient cultivars.

Triticale cultivar samples sustained evaluation for drought tolerance under control conditions (distilled H_2O) and varying concentrations of PEG (Table 4). The drought tolerance index (DTI) and the damage index (DI) incurred formulations based on the shoot length (SL). With higher PEG concentrations (20%–25%), the seedling growth showed a significant reduction across all the cultivars, confirming the inhibitory effect of osmotic stress. In the 20% PEG solution, the triticale cultivars with the highest DTI were the Sergiy

(50.12%), Xlebarob (45.18%), and K. Prag (34.85%). However, with 25% PEG solution, the highest DTI values resulted in the cultivars Sergiy (16.82%), Xlebarob (15.88%), and K. Prag (10.23%) (Table 4). The DI for these cultivars was lower than others. These results proved crucial for identifying the triticale cultivars best suited for cultivation under drought stress conditions and for further guiding breeding strategies. Khan *et al.* (2019) observed PEG-induced osmotic stress reduces plant growth; however, some wheat genotypes showed greater resistance. Their findings indicated lower damage indices in stress conditions were visible in the cultivars Sergiy and Xlebarob, which signified the highest adaptability to arid environments.

The varying concentrations of PEG-6000 exerted differential effects on the growth dynamics of triticale roots and shoots (Table 5). This study aimed to identify factors influencing triticale cultivars' growth and development under drought stress conditions. The differences in root and shoot length between the control (0% PEG) and 25% PEG reached computations, revealing significant variations in drought stress sensitivity among the triticale cultivars. The most sensitive cultivars to drought stress were PRAG-48/1, with a root length reduction of 87.14% and shoot length reduction of 97.04%, and Do'stlik-4, with a root length reduction of 66.34% and shoot length reduction of 95.97%. These cultivars exhibited the most sensitivity to drought stress conditions. The least affected triticale cultivars, demonstrating the highest drought tolerance, were Sergiy, with a root length reduction of 52.95% and shoot length reduction of 83.18%, and Xlebarob, with a root length reduction of 56.70% and shoot length reduction of 84.12%. These cultivars maintained relative stability under drought stress conditions. Ahmed *et al.* (2021) reported drought-sensitive wheat genotypes showed noteworthy root and shoot reductions within stress conditions, aligning with triticale cultivars PRAG-48/1 and Do'stlik-4 in this study. Conversely, cultivars Sergiy and Xlebarob demonstrated adaptive growth strategies, supporting their considerable drought tolerance.

The presented results highlighted the drought tolerance of triticale cultivars Sergiy and Xlebarob, while in contrast, the cultivars PRAG-48/1 and Do'stlik-4 showed high sensitivity, and the cultivar K. Prag exhibited moderate drought tolerance. These findings provide valuable insights for breeding programs. The results were consistent with past studies carried out by Chaves *et al*. (2002), who evaluated physiological responses in plants under simulated drought conditions using PEG, demonstrating that PEG-6000 effectively mimics strong osmotic stress. Furthermore, the highest PEG concentrations appeared to severely restrict the plant's root growth, a phenomenon observed in particular triticale cultivars in this study. Siddique *et al.'s* (2017) findings detailed that PEG-induced stress highlights genotypic differences in cereal crops for drought tolerance, with the same observed in the triticale cultivars Sergiy and Xlebarob. Their study further revealed that moderate tolerance, as observed in the cultivar K. Prag, can also gain enhancements through targeted breeding efforts.

Molecular analysis

Based on the results, 11 DNA markers associated with drought tolerance succeeded in their evaluation using PCR, identifying six of these markers as polymorphic. Among the polymorphic markers, four exhibited a polymorphism information content (PIC) value greater than 0.5. The primer pairs Xgwm484 (0.68) and Wmc525 (0.71) demonstrated the highest levels of polymorphism (Table 6). Li *et al.* (2022) reported elevated PIC values reflect substantial genetic diversity in cereals, supporting the effectiveness of primers Xgwm484 and Wmc525 in identifying drought tolerance. Their research advocates the use of such markers in molecular breeding to improve crop resilience.

According to the PCR analysis, molecular evaluation of selected triticale samples based on polymorphic markers associated with drought tolerance revealed the primer Xwmc78 DNA produced a total of four alleles with 250, 280, 300, and 315 bp from 10 triticale cultivar samples. Cultivars K. Prag,

Table 5. Number of roots, root length, and shoot length for each cultivar at control and 5%, 10%, 15%, 20%, and 25% PEG-6000 concentrations.

		Control (0% PEG)			PEG-6000 5%			PEG-6000 10%			PEG-6000 15%			PEG-6000 20%			PEG-6000 25%		
No,	Cultivars	Number of Roots	Root Length (cm)	Shoot Length (cm)	Number of Roots	Root Length (cm)	Shoot Length (cm)	Number of Roots	Root Length (cm)	Shoot Length (cm)	Number of Roots	Root Length (cm)	Shoot Length (cm)	Number of Roots	Root Length (cm)	Shoot Length (cm)	Number of Roots	Root Length (cm)	Shoot Length (cm)
1	Do'stlik-4	5.00±0.19	13.07±0.26	10.43±0.7	4.67±0.35	11.06±0.47	7.53±0.75	4.01±0.37	9.12±0.71	6.15±0.92	4.00±0.22	7.58±0.56	5.19±0.80	3.79±0.31	7.26±0.58	2.56±0.37	2.57±0.41	4.40±0.68	0.42±0.21
2	Tixon	5.17±0.29	13.76±0.99	11.97±0.88	5.00±0.21	11.97±0.78	9.24±0.84	4.23±0.26	10.02±0.53	7.51±0.66	4.16±0.31	8.57±0.63	6.82±0.94	4.00±0.31	8.02±0.53	3.61±0.35	3.75±0.40	5.26±0.86	1.02±0.39
3	Kunak	5.12±0.31	13.03±1.12	11.84±0.55	4.33±0.17	10.57±0.84	7.87±0.39	4.00±0.21	9.03±0.57	6.67±0.51	3.59±0.18	7.37±0.91	5.71±0.47	3.28±0.18	7.04±0.99	2.94±0.41	2.05±0.38	4.09±0.56	0.43±0.33
4	Yarillo	5.07±0.20	13.84±1.19	12.13±0.49	5.02±0.18	12.16±0.72	9.98±0.62	4.52±0.31	10.20±0.63	7.73±0.47	4.50±0.19	8.86±0.56	7.06±0.46	4.06±0.35	8.13±0.38	3.95±0.41	3.78±0.37	5.46±0.85	1.09±0.21
5	Sardor	5.40±0.21	14.46±0.84	15.13±1.02	5.03±0.24	13.25±0.69	13.53±0.75	4.87±0.23	11.17±0.42	10.03±0.63	4.60±0.25	10.01±0.61	8.98±0.71	4.19±0.17	9.19±0.61	5.01±0.39	4.00±0.48	5.79±0.80	1.45±0.3
6	K.Prag	5.43±0.16	14.96±0.92	15.15±0.96	5.06±0.18	13.97±0.48	13.82±0.63	4.97±0.18	11.81±0.69	10.52±0.55	4.80±0.27	11.04±0.8	9.35±0.64	4.32±0.27	9.79±0.84	5.28±0.4	4.01±0.53	6.33±0.78	1.55±0.43
7	Sergiy	5.66±0.16	15.41±0.70	12.13±0.56	5.43±0.24	15.02±0.80	11.99±0.50	5.14±0.28	12.85±0.57	9.14±0.46	5.06±0.25	12.05±0.49	8.83±0.57	4.93±0.24	10.34±0.35	6.08±0.53	4.13±0.54	7.25±0.84	2.04±0.29
8	Xlebarob	5.55±0.23	14.99±0.85	12.97±0.66	5.11±0.21	14.15±0.94	12.56±0.56	5.01±0.23	12.22±0.82	9.34±0.32	5.04±0.31	11.41±0.73	8.44±0.58	4.52±0.27	9.95±0.57	5.86±0.65	4.03±0.31	6.49±0.73	2.06±0.61
9	PRAO-02	4.77±0.23	13.38±1.14	14.00±0.57	4.71±0.23	11.42±0.87	10.56±0.74	4.13±0.22	9.58±0.50	8.41±0.61	4.09±0.21	8.17±0.83	7.81±0.45	3.83±0.31	9.17±0.90	4.17±0.42	2.97±0.41	4.65±0.52	0.75±0.06
10	PRAG-48/1	4.99±0.27	12.99±0.80	11.17±0.72	4.07±0.35	10.49±0.48	7.16±0.56	4.00±0.30	8.61±0.52	6.03±0.57	3.40±0.21	7.27±0.62	5.14±0.72	3.09±0.34	6.07±0.95	2.04±0.61	2.01±0.40	1.67±0.42	0.33±0.28

Table 6. SSR markers and their polymorphism information content (PIC) and heterozygosity (He) values.

No.	DNA markers	Molecular weight (base pairs, bp)	Specific bp.	PIC	He
1	Xgwm484	205, 225, 255	255	0.53	0.64
2	Wmc525	230, 245, 260, 290	230	0.72	0.74
3	Xgwm389	260, 280	260	0.45	0.66
4	Xwmc78	250, 280, 300, 315	280	0.43	0.45

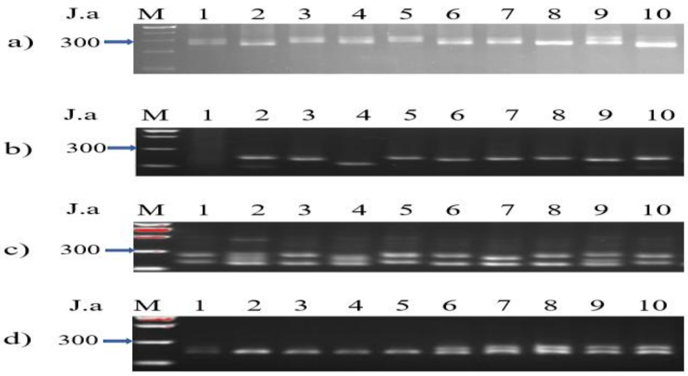

Figure 1. Electropherogram of PCR amplicons for polymorphic DNA markers associated with drought tolerance in triticale samples. M - Molecular weight marker. J.a (bp) - base pairs. 1 - Do'stlik-4, 2 - Tixon, 3 - Kunak, 4 - Yarillo, 5 - Sardor, 6 - K. Prag, 7 - Sergiy, 8 - Xlebarob, 9 - Prao-02, and 10 - Prag-48/1a-Xwmc78, b-Xgwm484, c-Xmc525, d-Xgwm389.

Sergly, Xlebarob, and Prao-02 emerged to possess the 280 bp allele of the DNA marker, which has an association with drought tolerance in these genotypes. In contrast, the drought tolerance allele of the primer Xwmc78 DNA was non-appearing in other triticale cultivars. However, within the selected samples, the presence of alleles (250, 300, and 315 bp) showed no association with drought tolerance (Figure 1). Zhang *et al.* (2020) identified specific alleles linked to stress resistance in wheat genotypes, including those similar to the 280 bp allele observed in the concerned study. Their findings emphasized the utility of allele-specific markers in recognizing drought-tolerant genotypes, validating the current results.

For the DNA primer Xgwm484, alleles of 205, 225, and 255 bp were notable in triticale cultivar samples during the drought tolerance assessment. In eight selected samples (Tixon, Kunak, Sardor, K. Prag, Sergiy, Xlebarob, Prao-02, and Prag-48/1), the drought tolerance allele seemed to be 255 bp. Among these triticale cultivar samples, Sardor, K. Prag, Sergiy, and Xlebarob appeared as homozygous for this allele. However, only one cultivar (Yarillo) exhibited a 225 bp allele, showing no connections with drought tolerance. The triticale cultivar Do'stlik-4 showed no allele for the Xgwm484 marker, indicating either the absence of the marker or an unsuccessful PCR process (Figure 1b). Wang *et al.* (2018) demonstrated that homozygous alleles enhanced the traits expression in cereals, explaining the drought tolerance in cultivars Sardor, K. Prag, Sergiy, and Xlebarob. They also reported PCR failure can result in missing alleles, consistent with the observation in the cultivar Do'stlik-4 used in this study.

For the DNA marker Xmc525, alleles of 230, 245, 260, and 290 bp were evident; however, the allele associated with drought tolerance was apparently 230 bp. This allele was noticeable in the triticale cultivars Tixon, Kunak, Yarillo, K. Prag, Sergiy, Xlebarob, and Prao-02, and these genotypes also exhibited morphological drought tolerance. Given this correlation with morphological traits, the reliability of the marker Xmc525 received a high rating (Figure 1c). Chen *et al.'s* (2019) findings enunciated that markers correlating with morphological traits in cereals, such as primer Xmc525, proved reliable in breeding programs. Their study further underscores the importance of such correlation in selecting drought-resistant genotypes, supporting the current results.

On the DNA primer Xgwm389, alleles of 260 and 280 bp appeared, and the drought tolerance allele was 260 bp, as confirmed to be present in all the triticale cultivars. The allele with 280 bp resulted in the genomes of six triticale cultivars (Figure 1d). Liu *et al.* (2021)

noted that ubiquitous alleles like 260 bp, which indicated widespread tolerance traits in wheat, were consistent with their presence across all the samples. They also suggested that secondary alleles, i.e., 280 bp, reflect genetic diversity, aligning with the observations in this study.

Analysis revealed four polymorphic DNA markers associated with drought tolerance, confirming that triticale cultivars K. Prag, Sergiy, and Xlebarob were evidently drought-tolerant based on both morphological and molecular assessments. Based on the PIC and heterozygosity (He) values, the primer Xmc525 emerges as the most reliable marker for future research. Yang *et al.'s* (2023) findings indicated that high PIC and He values enhanced the marker reliability, justifying the selection of primer Xmc525 in various crops. Their results supported the integration of morphological and molecular approaches for identifying drought-tolerant cultivars, as demonstrated in the presented study.

Using allelic profiles of the nine DNA markers linked to drought tolerance, the phylogenetic relationships among triticale cultivar samples underwent analysis with the MEGA 11 software. The clustering analysis highlighted genetic similarities and differences among the triticale cultivars (Figure 2). Kumar *et al.* (2020) utilized similar molecular approaches to assess the genetic relationship, validating the MEGA 11 analysis employed here. Their findings confirmed that such molecular analysis accurately reflects the genetic relatedness among the cereal crop cultivars.

The cluster analysis conducted to evaluate the drought tolerance of the triticale cultivars proved of significant importance for comparing their drought stress resistance. Through cluster analysis, triticale cultivars entailed groupings based on genetic and phenotypic similarities, with drought tolerance levels observed to increase from low to high. Patel *et al.* (2022) established that cluster analysis effectively categorizes cereal genotypes by similarity, supporting the observed tolerance gradient in this study. Their methodology reinforces the reliability of grouping tolerant and sensitive cultivars of the cereals.

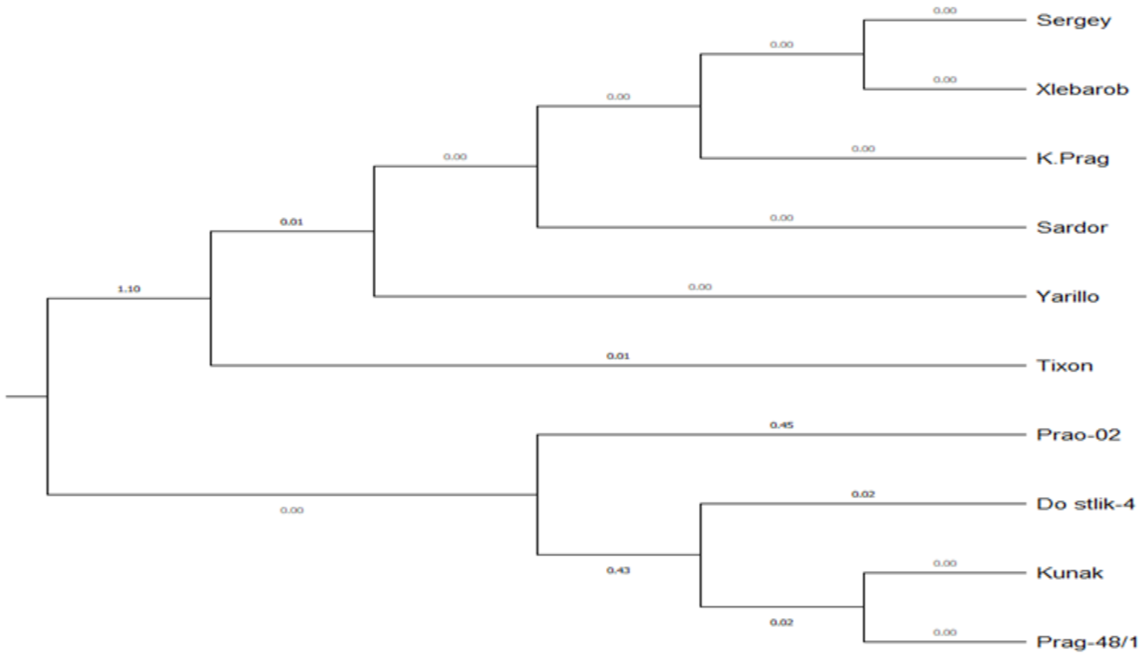

Figure 2. Phylogenetic tree of triticale samples based on polymorphism of DNA markers associated with drought tolerance alleles.

Based on the dendrogram analysis, the triticale cultivars with the lowest drought tolerance (Prag-48/1, Kunak, Do‵stlik-4, and Prao-02) formed a closely related cluster. These cultivars exhibited limited adaptability to drought stress conditions. Triticale cultivars with moderate drought tolerance (Tixon, Yarillo, and Sardor) displayed higher adaptability than the previous group, maintaining moderate productivity under dry climatic conditions. The most drought-tolerant triticale cultivars (Sergiy, Xlebarob, and K. Prag) created a distinct cluster, effectively sustaining growth and development under water-deficient conditions. Sharma *et al.* (2021) used dendrogram analysis to classify wheat genotypes by tolerance levels, supporting the clustering observed in this study. They highlighted that such classification aids in planning selective breeding strategies, consistent with the present recommendations made for the triticale cultivars Sergiy, Xlebarob, and K. Prag.

The relevant research findings hold crucial importance for future breeding programs to enhance triticale productivity under drought stress conditions. The study recommends the highly drought-tolerant triticale cultivars (Sergiy, Xlebarob, and K. Prag) for cultivation in the arid regions. Cultivars with moderate and low drought tolerance require inclusion in breeding programs, either through genetic improvement or by incorporating drought-tolerant genotypes. Rao *et al.* (2023) recommended cultivating tolerant cultivars like Sergiy, Xlebarob, and K. Prag in arid regions for sustainable production. They also emphasized genetic enhancement for less tolerant cultivars, supporting the breeding strategies proposed in this valuable study.

CONCLUSIONS

The opportune study revealed considerable drought tolerance in triticale cultivars under 20% and 25% PEG-6000 stress conditions, and cultivars Sergiy, Xlebarob, and K. Prag achieved a superior germination rate (96.7%). The DNA markers Xgwm484 (255 bp allele), Xmc525 (230 bp allele), and Xgwm389 (260 bp allele) confirmed their significant drought tolerance, highlighting their reliability for breeding. Phylogenetic analysis further elucidated the genetic relationship among the triticale cultivars, emphasizing the role of these markers in enhancing breeding outcomes. These triticale cultivars proved promising candidates for developing drought-resistant cultivars through future breeding programs.3

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