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Polycross breeding enhances cumin quality and drought tolerance for sustainable agriculture

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The yield and quality of cumin (*Cuminum cyminum* L.) medicinal plant are significantly affected by water stress. Producing a synthetic variety of cumin using polycross breeding can be an appropriate method for optimizing the biosynthesis of metabolites to create high-yielding, drought-tolerant plants with higher metabolite content and antioxidant properties. Therefore, a comprehensive study was conducted to evaluate the impact of drought stress on seed yield, metabolite content, antioxidant properties of ethanolic, methanolic, and aqueous extracts, essential oil content and composition, fatty acid composition, and physical seed traits of the synthetic variety of cumin in comparison with several parental genotypes over two growing seasons under normal irrigation (100% field capacity) and drought stress (30% field capacity) conditions. The results showed a 79.58%, 12.78% and 12.89% increase in seed yield, protein and carbohydrate content of the synthetic cultivar compared to the average of the parental genotypes under drought stress conditions. The decreasing effect of drought stress on the physical traits of seeds including 1000-seed weight, area, circumference, length and width of the seed in the synthetic cultivar (6.31, 20.84, 17.56, 9.44 and 7.45% respectively) was much less than that of the parental genotypes (9.67, 31.82, 18.56, 19.76 and 11.99% respectively). Under drought stress conditions, the synthetic cultivar had the highest essential oil content (3.11%) and oil content (12.04%). Also, in the synthetic variety, the amount of the main active compound of the essential oil (cumin aldehyde) and oil (oleic acid) also increased more due to drought stress. The synthetic variety had higher content of secondary metabolites (phenol, flavonol and flavonoid) and antioxidant properties, especially under drought stress conditions. Ethanol was also identified as the most suitable solvent for the extraction of polyphenolic compounds and antioxidant power. These findings suggest that producing a synthetic variety can be a suitable option for breeding high-quality cumin in arid regions. The results of this study can be utilized in breeding programs to develop drought-tolerant cumin varieties and other related plants with high yield and quality.

Keywords *Cuminum cyminum*, Drought, Synthetic variety, Polyphenol, Protein, Sugar

Drought is one of the most significant environmental stresses and a limiting factor for successful crop production worldwide, especially in arid and semi-arid regions[1]. Water scarcity not only reduces vegetative growth and alters plant anatomical structure but also triggers oxidative stress, which in turn induces changes in the biosynthetic pathways of various secondary metabolites[2]. Plant adaptation to drought stress is the result of changes in various morphological, physiological, and phytochemical mechanisms, leading to alterations in plant growth rate, stomatal conductance, photosynthesis process rate, and enzyme activities[3]. One of the mechanisms plants use under drought stress conditions is maintaining intracellular osmotic potential by accumulating soluble sugars and amino acids[4], which leads to the stability of cell membranes and maintenance of cell turgor[5]. On the other hand, the accumulation of reactive oxygen species (ROS) in cells can damage membrane lipids, proteins, and nucleic acids[6,7]. Plants deploy antioxidant defense mechanisms to counteract ROS effects, including enzymatic systems like GPOD and APX[8]. Additionally, non-enzymatic defense mechanisms involving the accumulation of polyphenolic compounds (phenols, flavonoids, flavones, flavanones, isoflavones, flavonols, catechins, flavans, biflavans, tannins, etc.), and anthocyanins play a crucial role in neutralizing ROS and enhancing drought tolerance in plants[9].

Polyphenolic compounds, due to their antioxidant activity, prevent lipid peroxidation and oxidative modification of lipoproteins[10]. These compounds act as free radical scavengers and contribute to plant resistance

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against oxidative stresses[11]. Drought tolerance is achieved by increasing the levels of antioxidants to eliminate ROS, as confirmed by other researchers' reports[8].

Plants with higher levels of antioxidants generally show greater resistance to oxidative damage. The presence of polyphenolic compounds is directly involved in antioxidative action[12]. Numerous studies have been conducted on antioxidant compounds, and even some synthetic antioxidants have been marketed, which are limited in use due to toxicity. Therefore, finding natural antioxidants, especially from plants, and using them, particularly in the food and pharmaceutical industries, is highly desirable. In addition to having broad biological effects, the likelihood of side effects and toxicity, especially at controlled concentrations, is reduced[13]. In the process of extracting polyphenols, to recover maximum yield and the highest quality (concentration of target compounds and antioxidant power of extracts), different solvent systems such as water and organic solvents like ethanol, methanol, acetone, and diethyl ether must be used[14,15].

Cumin (*Cuminum cyminum* L.) is a widely cultivated industrial and medicinal plant, predominantly grown in arid and semi-arid regions such as the Middle East, India, China, and the Mediterranean areas. Farmers favor its cultivation due to its short growing season of 100–120 days, low water requirements, and high economic value. This plant is a rich reservoir of many biologically active compounds with various therapeutic applications[16,17]. Among its parts, cumin seeds are of utmost importance due to their high concentration of biologically active compounds, making them a valuable source of medicinal compounds and natural antioxidants[18]. The seeds contain volatile oil (5%), fat (22%), protein (10%), fiber (11%), fixed oil (10%), free amino acids, protein, cellulose, sugar, and minerals[19].

Despite being adapted to dry conditions and its vegetative growth period coinciding with winter and spring rains, cumin is affected by drought stress during the reproductive and seed-filling stages (end-of-season drought). Although end-of-season drought stress improves the quality of this plant, it severely affects seed yield and reduces seed performance. Therefore, breeding drought-tolerant cumin varieties can help mitigate the adverse effects of drought stress and increase the metabolic efficiency and antioxidant properties of this medicinal plant. Drought tolerance is defined as a mechanism that causes the least reduction in yield under stress conditions compared to the highest yield in a well-water environment. A drought-tolerant variety quickly perceives drought stress and activates signal transduction pathways to stimulate downstream components to resist drought stress. Thus, the optimal performance of polyphenols and antioxidant properties can be achieved using a drought-tolerant variety.

Cumin has hermaphroditic flowers with protandrous nature, making it an open-pollinating plant. The development of superior varieties in open-pollinating plants often involves hybridization and a rigorous screening process, achieved through the crossing of superior genotypes in classical breeding[20]. Hybrid breeding primarily benefits from heterosis, or hybrid vigor[21]. However, breeding for hybrid production necessitates the use of an efficient pollination control system, such as cytoplasmic male sterility. If such a system is not available, breeding synthetic varieties through the polycross test can be a suitable option to utilize heterosis. Synthetic varieties are of breeding varieties generated by random-mating of a limited number of selected superior genotypes as parents in a polycross treasury, based on the general combining ability of important traits[22]. Polycross testing has been successfully used for genetic improvement of the medicinal plants *Artemisia scoparia*, *Salvia miltiorrhiza*, *Leonurus japonica*, and *Thymus daenensi*[23]; And synthetic varieties, characterized by their high heterozygosity compared to parental genotypes, exhibit greater adaptability and stability in different environments. Several reports have shown that synthetic varieties maintain primary genetic diversity and evolve in response to varying conditions, resulting in high performance under stress conditions, making them suitable for challenging environments like arid regions[24,25]. To the best of our knowledge, no study has been conducted on creating a drought-tolerant breeding variety to improve the quantity and quality of cumin. Therefore, the present study aims to investigate the effect of drought stress on morphological and phytochemical traits. Additionally, we aim to evaluate different solvent systems impact on the quantities of metabolic traits, specifically polyphenolic compounds, and antioxidant activity of seeds of a synthetic drought-tolerant variety of cumin and its parental genotypes.

Results

The analysis of variance revealed a significant difference between the parental genotypes and the progeny of the synthetic variety in relation to all studied traits (Table 1). The superiority performance of the first (SYN1) and second generation (SYN2) progeny of the synthetic variety, compared to the parental genotypes, under both irrigation conditions, highlights not only the combined genetic effects of the parent genotypes during random polycross mating and the heritability of traits in the progeny but also emphasizes the advantages and applications of the synthetic variety. Furthermore, the interaction between genotype and moisture conditions for the measured traits was also significant. However, the interaction between location and moisture conditions, as well as the interaction of genotype by location, were not significant. This suggests that the studied genotypes exhibit consistent performance across different environments, suggesting a level of stability in trait expression. The studied traits are primarily controlled by genetic effects and are less influenced by environmental conditions, indicating genetic uniformity, which is a desirable characteristic of varieties. Therefore, the mean of the two locations was used for statistical calculations.

Considering the proximity to the maximum yield potential in the second generation (SYN2) of the synthetic variety[26], and the lack of a significant difference between the first generation (SYN1) and second generation (SYN2) of the synthetic variety under normal irrigation conditions, as well as the inability to compare the first generation (SYN1) with the parental genotypes under drought stress conditions, this study only discussed the results of the second generation (SYN2) of the synthetic variety.

			Condition (C)	Year (Y)	Genotype (G)	Y× C	G× C	G×Y	CV%
Yield			**	ns	**	ns	**	ns	8.95
Protein			**	ns	**	ns	**	ns	1.21
Sugar			**	ns	**	ns	**	ns	0.28
Essential oil			**	ns	**	ns	**	ns	2.12
Essential oil Yield			**	ns	**	ns	**	ns	11.48
Phenol		Ethanol	**	ns	**	ns	**	ns	2.42
		Methanol	**	ns	**	ns	**	ns	0.18
		Water	**	ns	**	ns	**	ns	0.61
Flavonoid		Ethanol	**	ns	**	ns	**	ns	0.16
		Methanol	**	ns	**	ns	**	ns	4.51
		Water	**	ns	**	ns	**	ns	0.09
Flavanol		Ethanol	**	ns	**	ns	**	ns	0.40
		Methanol	**	ns	**	ns	**	ns	0.17
		Water	**	ns	**	ns	**	ns	0.19
DPPH	25 µg/ml	Ethanol	**	ns	**	ns	**	ns	0.57
		Methanol	**	ns	**	ns	**	ns	0.21
		Water	**	ns	**	ns	**	ns	0.20
	50 µg/ml	Ethanol	**	ns	**	ns	**	ns	0.13
		Methanol	**	ns	**	ns	**	ns	3.09
		Water	**	ns	**	ns	**	ns	0.13
	90 µg/ml	Ethanol	**	ns	**	ns	**	ns	0.07
		Methanol	**	ns	**	ns	**	ns	0.07
		Water	**	ns	**	ns	**	ns	0.16
	130 µg/ml	Ethanol	**	ns	**	ns	**	ns	0.16
		Methanol	**	ns	**	ns	**	ns	0.12
		Water	**	ns	**	ns	**	ns	0.07
Reducing Power	100 µg/ml	Ethanol	**	ns	**	ns	**	ns	2.69
		Methanol	**	ns	**	ns	**	ns	8.03
		Water	**	ns	**	ns	**	ns	2.03
	300 µg/ml	Ethanol	**	ns	**	ns	**	ns	1.54
		Methanol	**	ns	**	ns	**	ns	1.75
		Water	**	ns	**	ns	**	ns	1.51
	600 µg/ml	Ethanol	**	ns	**	ns	**	ns	0.79
		Methanol	**	ns	**	ns	**	ns	1.71
		Water	**	ns	**	ns	**	ns	1.05
	900 µg/ml	Ethanol	**	ns	**	ns	**	ns	0.71
		Methanol	**	ns	**	ns	**	ns	0.71
		Water	**	ns	**	ns	**	ns	0.66

Table 1. Analyses of variances for different traits of cumin. **Significant at the 1% level, Ns indicates statistically non-significant at the 5% level.

Seed yield

The average seed yield per square meter under drought conditions decreased from 166.06 to 101.65 g in the mean of the parental genotypes (Table 2). The seed yield of the synthetic cumin variety (SYN2) under normal irrigation and drought conditions was 229.92 and 182.55 g per square meter, respectively, showing a superiority of 38.46% and 79.58% over the mean of the parental genotypes. Furthermore, the yield reduction due to drought stress decreased from 63.37% in the mean of the parental genotypes to 25.95% in the synthetic variety (SYN2).

Primary metabolites

Seed protein content

The crude protein content of cumin seeds ranged from 19.25 to 23.49% under normal irrigation condition and from 21.63 to 24.93% under drought stress condition (Table 2). The synthetic cultivar (SYN2) exhibited the highest protein content under both normal and drought stress conditions. The increase in protein content of the synthetic cultivar (SYN2) compared to the average of the parental genotypes was 11.26% under normal irrigation condition and 12.78% under drought stress condition.

Condition		Yield (g m^2)	Protein (%)	Suger	Phenol (mg GAE g − 1 DW)			Flavonoid (mg CE g − 1 DW)			Flavonol (mg CE g − 1 DW)		
					Ethanol	Methanol	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Water
Normal	SYN1	254.71	23.49	1.11	35.53	33.97	24.03	54.03	53.50	39.63	26.46	25.50	21.66
	SYN2	229.92 ± 3.23a	23.34 ± 0.0007a	1.08 ± 0.007a	34.43 ± 0.01a	33.49 ± 0.08a	23.53 ± 0.01a	53.49 ± 0.10a	52.23 ± 0.10a	38.26 ± 0.004a	25.49 ± 0.09a	25.13 ± 0.02a	21.55 ± 0.01a
	YAR1	183.23 ± 25.64b (25.48)	21.88 ± 0.06b (6.68)	0.93 ± 0.001d (16.34)	32.17 ± a2.92b (7.01)	29.71 ± 0.05d (12.75)	17.24 ± 0.001d (36.54)	41.29 ± 0.16e (29.54)	34.14 ± 0.009c (52.98)	22.02 ± 0.01c (73.81)	18.42 ± 0.02c (38.37)	17.65 ± 0.01c (42.38)	16.12 ± 0.005c (33.69)
	GJA3	176.79 ± 16.95b (30.05)	20.70 ± 0.02c (12.80)	0.91 ± 0.005e (18.68)	27.18 ± 0.06c (26.65)	26.02 ± 0.21e (28.73)	15.69 ± 0.23e (49.95)	47.61 ± 0.11b (12.35)	43.91 ± 0.01b (18.95)	35.42 ± 0.02f (8.04)	16.96 ± 0.08d (50.31)	15.51 ± 0.07d (62.00)	14.29 ± 0.008e (50.81)
	KRA5	128.28 ± 6.11c (79.23)	22.16 ± 0.33b (5.35)	0.90 ± 0.003e (19.51)	23.23 ± 0.02d (48.22)	22.23 ± 0.07f (50.65)	11.97 ± 0.26f (96.57)	44.60 ± 0.005d (19.93)	41.40 ± 0.78b (26.15)	33.71 ± 0.03d (13.50)	15.65 ± 0.07e (62.90)	15.11 ± 0.02f (66.26)	13.08 ± 0.06f (64.69)
	SKD6	173.03 ± 3.74b (32.88)	19.25 ± 0.57d (21.24)	0.97 ± 0.004b (11.43)	31.28 ± 0.007b (10.05)	30.63 ± 0.09c (9.34)	19.15 ± 0.03c (22.91)	45.08 ± 0.04c (18.65)	39.07 ± 0.76b (33.68)	35.70 ± 0.07b (7.19)	16.78 ± 0.40d (51.91)	15.39 ± 0.02e (63.32)	14.76 ± 0.06d (46.00)
	NKM9	168.98 ± 8.40b (36.07)	20.92 ± 0.09c (11.61)	0.95 ± 0.001c (13.76)	32.15 ± 0.01ab (7.09)	31.45 ± 0.01b (6.49)	21.20 ± 0.04b (11.02)	40.37 ± 0.11f (32.49)	40.96 ± 0.01b (27.50)	27.17 ± 0.07e (40.81)	20.22 ± 0.21b (26.05)	19.72 ± 0.03b (27.42)	17.72 ± 0.05b (21.60)
	Parental Mean	166.06 (38.46)	20.98 (11.26)	0.93 (15.86)	29.20 (17.89)	28.01 (19.58)	17.05 (38.03)	43.79 (22.15)	39.90 (30.91)	30.80 (24.22)	17.61 (44.78)	16.68 (50.68)	15.19 (41.82)
Stress	SYN1	–	–	–	–	–	–	–	–	–	-	–	–
	SYN2	182.55 ± 4.77	24.93 ± 0.009	1.28 ± 0.002	42.28 ± 0.01	39.03 ± 0.01	28.32 ± 0.19	59.83 ± 0.007	61.23 ± 0.006	44.32 ± 0.002	30.14 ± 0.007	29.41 ± 0.007	21.88 ± 0.008
	YAR1	137.23 ± 14.10 (33.02)	21.79 ± 0.38 (14.40)	1.08 ± 0.005 (18.36)	34.87 ± 0.96 (21.24)	33.61 ± 0.006 (16.10)	21.70 ± 0.004 (30.50)	51.61 ± 0.001 (15.93)	44.30 ± 0.002 (38.22)	27.03 ± 0.03 (63.97)	20.62 ± 0.004 (46.19)	20.14 ± 0.002 (46.03)	18.84 ± 0.004 (16.10)
	GJA3	129.24 ± 21.35 (41.24)	21.85 ± 0.08 (14.10)	1.05 ± 0.002 (22.21)	33.14 ± 0.003 (27.56)	31.20 ± 0.002 (25.07)	18.70 ± 0.006 (51.50)	54.91 ± 0.01 (8.96)	51.90 ± 0.003 (17.98)	42.21 ± 0.02 (4.99)	18.24 ± 0.003 (65.24)	17.85 ± 0.002 (64.78)	15.54 ± 0.004 (40.83)
	KRA5	61.74 ± 9.57 (195.68)	22.46 ± 0.11 (11.02)	1.08 ± 0.005 (17.86)	27.93 ± 0.004 (51.34)	26.59 ± 0.001 (46.74)	15.80 ± 0.001 (79.22)	56.20 ± 0.003 (6.46)	51.73 ± 2.30 (18.37)	41.02 ± 0.03 (8.04)	16.92 ± 0.01 (78.19)	16.50 ± 0.004 (78.23)	14.60 ± 0.007 (49.82)
	SKD6	117.00 ± 0.95 (56.02)	22.79 ± 0.28 (9.38)	1.23 ± 0.001 (3.50)	35.42 ± 0.002 (19.35)	30.99 ± 0.04 (25.92)	19.71 ± 0.02 (43.68)	49.80 ± 0.005 (20.14)	46.01 ± 0.01 (33.10)	36.50 ± 0.001 (21.43)	17.82 ± 0.01 (69.19)	17.32 ± 0.003 (69.77)	13.57 ± 0.003 (61.26)
	NKM9	63.04 ± 9.75 (189.59)	21.63 ± 0.27 (15.24)	1.22 ± 0.001 (5.12)	35.40 ± 0.004 (19.42)	34.20 ± 0.004 (14.11)	24.17 ± 0.37 (17.21)	50.21 ± 0.001 (19.16)	46.80 ± 0.005 (30.84)	29.40 ± 0.003 (50.76)	22.14 ± 0.005 (36.13)	21.70 ± 0.01 (35.51)	18.62 ± 0.005 (17.48)
	Parental Mean	101.65 (79.58)	22.10 (12.78)	1.13 (12.89)	33.35 (26.75)	31.32 (24.60)	20.02 (41.50)	52.54 (13.86)	48.15 (27.18)	35.23 (25.80)	19.15 (57.43)	18.70 (57.25)	16.23 (34.76)

Table 2. Yield, primary metabolite content (protein and sugar), and polyphenolic compounds of cumin. Different superscript letters (a–f) indicate significant differences ($p < 0.05$) among genotypes mean under the same treatment, based on Duncan's multiple range test. The values in parentheses indicate the percentage superiority of the synthetic cultivar compared to each parental genotype.

Seed sugar content

Environmental stress conditions have a significant impact on sugar metabolism. The results of this experiment revealed that reduced water potential led to an increase in soluble sugar content. Under normal irrigation condition compared to drought stress, the synthetic cultivar (SYN2) exhibited an increase in soluble sugar content from 1.08 to 1.28, while the average of the parental genotypes showed an increase from 0.93 to 1.13. This increasing trend was more pronounced in the synthetic cultivar (SYN2) compared to the parental genotypes (Table 2).

Secondary metabolites

Percentage, yield, and composition of essential oil

The percentage and yield of essential oil obtained from parental genotypes and the synthetic variety (SYN2) exhibited inconsistent results under different environmental conditions, and the percentage and yield of essential oil in drought stress conditions increased and decreased, respectively. Synthetic variety (SYN2) and the average of the parental genotypes displayed varying levels of essential oil percentage, with values of 2.74% and 1.14% under normal irrigation conditions, and 3.11% and 1.47% under water stress conditions (Table 3). Consequently, water stress in both the parents and the synthetic variety (SYN2) of cumin resulted in a 22.42% and 12.11% increase in essential oil content compared to normal irrigation conditions. In comparison to the average of the parental genotypes, the synthetic variety (SYN2) exhibited a remarkable improvement of 140.30% and 112.13% in essential oil content under normal irrigation and drought conditions, respectively. Also, the synthetic variety (SYN2) demonstrated the highest essential oil yield under both normal irrigation (692.16 g/m2) and water stress (568.64 g/m2) conditions (Table 2).

The essential oil composition of cumin can be classified into three groups: monoterpenes, sesquiterpenes, and sterols. Monoterpenes make up the majority, accounting for over 80% of cumin essential oil (Table 3). Cumin essential oil contains various monoterpenes, including beta-Pinene, p-Cymene, gamma-Terpinene, and Cumin aldehyde. Water stress has a positive and significant impact on the monoterpene compounds gamma-Terpinene and Cumin aldehyde. In comparison to the parental genotypes, the synthetic variety (SYN2) exhibited a higher percentage of the main active ingredient of cumin, Cumin aldehyde, under both irrigation conditions. The amount of Cumin aldehyde in the synthetic variety (SYN2) increased by approximately 13% under drought stress, rising from 67.02 to 77.22% compared to normal conditions.

Polyphenolic compounds

Quantitative assessment of polyphenolic compounds in different ethanol, methanol, and aqueous extracts showed that drought stress had a positive impact on the bioactive compounds present in cumin seeds (Table 2). The results indicated that water shortage in the synthetic variety (SYN2) led to increases in phenol, flavonol, and flavonoid contents in the extracts. Specifically, the ethanol extracts showed an increase from 34.43 to 42.28 in phenol content, from 25.49 to 59.83 in flavonol content, and from 53.49 to 30.14 in flavonoid content. Similarly, the methanol extracts exhibited an increase from 33.49 to 39.03 in phenol content, from 25.13 to 29.41 in flavonol content, and from 52.23 to 61.23 in flavonoid content. Lastly, the aqueous extracts showed an increase

Compound	Type of composition	Normal							Stress						
		SYN1	SYN2	YAR1	GJA3	KRA5	SKD6	NKM9	SYN1	SYN2	YAR1	GJA3	KRA5	SKD6	NKM9
beta-Pinene (C10H16)	Monoterpene	10.25	–	5.20	6.04	6.85	6.78	4.86	–	–	6.49	6.05	4.08	4.92	3.78
p-Cymene (C10H14)	Monoterpene	13.15	1.99	6.61	9.54	8.09	7.92	8.78	–	0.39	6.36	10.22	7.94	7.26	10.30
gamma-Terpinene (C10H16)	Monoterpene	5.41	5.40	6.79	3.43	7.47	8.81	5.15	–	3.14	8.25	2.31	2.60	9.37	5.86
Cumin aldehyde (C10H12O)	Monoterpene	60.57	67.02	62.44	66.76	60.34	55.24	57.48	–	77.22	59.69	54.77	57.33	62.80	61.71
cis-beta-Farnesene (C15H24)	Sesquiterpene	1.34	–	3.07	3.85	2.86	2.25	2.56	–	–	1.78	4.79	4.89	1.72	4.15
(+)-Acoradiene (C15H24)	Sesquiterpene	0.70	7.24	1.05	0.72	1.04	1.76	1.35	–	9.63	1.17	0.86	2.83	1.27	1.34
Carotol (C15H26O)	Sesquiterpene	0.21	2.45	0.95	1.28	1.19	1.12	1.05	–	3.27	1.07	1.05	1.02	0.52	0.37
Phthalic acid (C8H6O4)	Estetrol	2.20	3.47	2.72	1.50	1.53	1.04	1.23	–	5.96	3.68	2.09	5.29	1.21	1.63
Essential oil (%)		3.15	2.74	0.86 (217.80)	1.32 (107.63)	1.26 (117.05)	1.10 (148.82)	1.15 (137.08)	–	3.11	1.07 (192.04)	1.97 (58.11)	1.56 (100.05)	1.36 (128.35)	1.38 (125.03)
Essential oil Yield (g.m2)		803.19	629.12	157.83 (298.60)	233.13 (169.86)	161.72 (289.02)	190.31 (230.57)	195.66 (221.53)	–	568.64	146.29 (288.71)	255.03 (122.97)	96.30 (490.50)	159.56 (256.38)	87.17 (552.34)

Table 3. Essential oil content and composition of Cumin (*Cuminum cyminum* L.) seeds. The values in parentheses indicate the percentage superiority of the synthetic cultivar compared to each parental genotype.

from 23.53 to 28.32 in phenol content, from 21.55 to 21.88 in flavonol content, and from 38.26 to 44.32 in flavonoid content.

The synthetic variety (SYN2) exhibited a higher percentage improvement in secondary metabolites compared to the average of parental genotypes in all three types of extracts. The improvement in phenol content was estimated at 26.75% in ethanol extracts, 24.60% in methanol extracts, and 41.50% in aqueous extracts. Similarly, the improvement in flavonol content was estimated at 57.43% in ethanol extracts, 57.25% in methanol extracts, and 34.76% in aqueous extracts. Finally, the improvement in flavonoid content was estimated at 13.86% in ethanol extracts, 27.18% in methanol extracts, and 25.80% in aqueous extracts. Also, ethanol extracts exhibited the highest amounts of polyphenolic compounds under both normal irrigation and water stress conditions.

Antioxidant properties

The analysis of antioxidant activity using DPPH and reducing power methods showed a direct relationship between the levels of polyphenolic compounds and the antioxidant properties of cumin. The results demonstrated that under drought stress condition, the increase in polyphenolic compounds led to an increase in antioxidant activity (Tables 4 and 5). Moreover, the antioxidant activity of different extracts showed a logical increase with increasing extract concentration. Specifically, the ethanol extract of the synthetic variety (SYN2) exhibited the highest antioxidant activity under drought stress conditions at a concentration of 130 µg/mL (143.20) in the DPPH assay and at a concentration of 900 µg/mL (1.72) in the reducing power assay.

Physical seed traits

Moisture stress had a negative and reducing effect on the physical traits of seeds (Fig. 1). The percentage decrease in traits such as thousand-seed weight, area, perimeter, length, and width due to drought stress was 6.31%, 20.84%, 17.56%, 9.44%, and 7.45% for the synthetic variety (SYN2), and 9.67%, 31.82%, 18.56%, 19.76%, and 11.99% for the average of the parental genotypes, respectively. Thousand-seed weight exhibited a relatively lesser negative impact from drought stress compared to other physical traits, declining from 4.80 g to 4.51 g in the synthetic variety (SYN2) and from 3.47 g to 3.16 g in the average of the parental genotypes.

Cluster and principal component (PCA) analysis

To categorize parental genotypes and the cumin synthetic variety, cluster analysis was conducted using the Ward method with the squared Euclidean distance similarity coefficient. Based on cluster analysis, the synthetic variety was distinctly separated from the parental genotypes under both humidity conditions (Fig. 2). Additionally, principal component analysis (PCA) was performed to further explore and understand the relationship between the parental genotypes and the synthetic variety. The findings revealed that the first two components accounted for 99.36 and 99.29% of the variation between the parental genotypes and the synthetic cultivar under normal irrigation and moisture stress conditions, respectively. In the first component, polyphenol content and antioxidant properties had the highest influence, whereas the second component was most strongly associated with seed yield. The graphical presentation of the parental genotypes and synthetic variety in a two-dimensional plot, based on the first two principal components, confirmed the groupings derived from the cluster analysis (Fig. 3).

Percentage and composition of fatty acids

The oil content of cumin seeds exhibited an increase (%) under drought stress conditions. Under normal irrigation conditions, the oil percentage in the average of the parental genotypes and the synthetic variety (SYN2) was estimated at 5.48% and 8.78%, respectively. However, under drought stress conditions, these values rose to 7.26% and 12.04%, respectively. Notably, the synthetic variety (SYN2) consistently displayed a higher oil percentage than the parental genotypes under both normal irrigation and drought stress conditions (Table 6).

Upon analyzing the fatty acid compositions of cumin seeds, oleic acid and Linoleic acid were identified as the primary components (Table 6). Cumin seeds exhibited a high proportion of unsaturated fatty acids (UFA). In the synthetic variety (SYN2), the levels of polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) were 31.64% and 6.02%, respectively, under normal irrigation conditions, and 29.73% and 6.85%, respectively, under drought stress conditions.

Discussion

Drought stress significantly affects the metabolite content and antioxidant properties of medicinal plants and leads to complex biochemical adaptations. Increasing the metabolite content and antioxidant properties in plants is very important to increase their flexibility against oxidative stress and improve their nutritional value. Therefore, it is also important to consider potential trade-offs, such as energy costs associated with the production of these compounds, which may affect overall plant growth and performance. Production of drought-tolerant synthetic cultivars is a promising strategy to increase yield, metabolite content, and antioxidant properties in medicinal plants. Our results showed that placing superior parental genotypes in the random crossing of the polycross test and creating a synthetic variety increases the metabolic content and antioxidant activity of cumin in both normal irrigation conditions and water deficit stress compared to the parental genotypes which was able to improve the overall flexibility of the plant under drought stress conditions. While the production of drought-tolerant synthetic varieties of cumin is promising for increasing the production of bioactive compounds, no information about drought tolerance in metabolic traits and antioxidant properties of drought-tolerant varieties has been reported in this species. Therefore, it is necessary to discuss the complexity of drought tolerance mechanism in different target traits.

Condition		25 µg/ml			50 µg/ml			90 µg/ml			130 µg/ml		
		Ethanol	Methanol	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Water
Normal	SYN1	61.86	58.11	46.95	72.31	64.90	56.66	92.34	89.32	68.69	134.54	126.65	89.45
	SYN2	61.20 ± 1.32a	57.05 ± 0.23a	44.53 ± 0.03a	71.21 ± 0.13a	63.44 ± 0.02a	55.23 ± 0.14a	91.77 ± 0.13a	87.34 ± 0.29a	66.50 ± 0.001a	131.26 ± 0.007a	123.30 ± 0.28a	86.36 ± 0.23a
	YAR1	47.02 ± 0.009f (30.16)	43.54 ± 0.21f (31.02)	30.07 ± 0.05f (48.08)	58.53 ± 0.01c (21.67)	55.11 ± 0.03d (15.11)	42.50 ± 0.03c (29.94)	73.80 ± 0.005d (24.34)	71.41 ± 0.02d (22.30)	51.43 ± 0.03b (29.30)	92.34 ± 0.02e (42.14)	90.20 ± 0.07d (36.69)	68.32 ± 0.06d (26.40)
	GJA3	48.50 ± 0.001d (26.17)	46.33 ± 0.05c (23.13)	34.54 ± 0.09b (28.91)	55.28 ± 0.02c (28.83)	53.21 ± 0.08d (19.22)	40.63 ± 0.08c (35.91)	79.15 ± 0.004d (15.93)	76.19 ± 0.01d (14.63)	48.21 ± 0.2d (37.94)	111.24 ± 0.08e (17.99)	108.28 ± 0.14d (13.87)	72.59 ± 0.01d (18.96)
	KRA5	48.00 ± 0.16e (27.49)	46.12 ± 0.05d (23.69)	31.58 ± 0.07d (41.01)	58.22 ± 0.22d (22.32)	56.35 ± 0.008c (12.57)	35.57 ± 0.05f (55.25)	73.25 ± 0.01e (25.28)	71.46 ± 0.13d (22.23)	40.72 ± 0.09e (63.33)	99.51 ± 0.02c (31.90)	97.54 ± 0.05c (26.41)	72.55 ± 0.008c (19.02)
	SKD6	51.38 ± 0.03b (19.11)	48.35 ± 0.22b (17.99)	33.35 ± 0.10c (33.50)	61.19 ± 0.01b (16.38)	58.52 ± 0.008b (8.41)	43.37 ± 0.11b (27.35)	75.12 ± 0.006c (22.15)	73.48 ± 0.06c (18.86)	48.38 ± 0.24c (37.46)	111.41 ± 0.009b (17.81)	108.45 ± 0.11b (13.70)	64.81 ± 0.01e (33.25)
	NKM9	49.23 ± 0.06c (24.31)	45.21 ± 0.05e (26.19)	30.58 ± 0.22e (45.59)	58.64 ± 0.11c (21.43)	54.73 ± 0.76e (15.91)	38.36 ± 0.07e (43.97)	68.69 ± 0.08f (33.59)	64.44 ± 0.06e (35.54)	46.31 ± 0.02d (43.59)	93.71 ± 0.58d (40.07)	85.59 ± 0.08e (44.07)	78.30 ± 0.01b (10.28)
	Parental Mean	48.83 (25.34)	45.91 (24.26)	32.03 (39.04)	58.37 (22.00)	55.59 (14.13)	40.09 (37.77)	74.00 (24.00)	71.40 (22.33)	47.01 (41.46)	101.64 (29.13)	98.01 (25.80)	71.32 (21.09)
Stress	SYN1	–	–	–	–	–	–	–	–	–	–	–	–
	SYN2	66.29 ± 0.003	64.23 ± 0.006	43.58 ± 0.01	78.23 ± 0.004	75.27 ± 0.01	54.60 ± 0.005	100.50 ± 0.008	96.60 ± 0.004	69.50 ± 0.007	143.20 ± 0.005	129.17 ± 0.002	99.80 ± 0.004
	YAR1	51.38 ± 0.006 (29.04)	49.31 ± 0.003 (30.25)	32.25 ± 0.005 (35.13)	62.24 ± 0.006 (25.69)	58.11 ± 0.09 (29.54)	45.51 ± 0.008 (19.97)	81.53 ± 0.003 (23.26)	78.73 ± 0.05 (22.70)	52.48 ± 0.007 (32.43)	111.50 ± 0.005 (28.43)	102.32 ± 0.005 (26.24)	81.20 ± 0.003 (22.90)
	GJA3	49.23 ± 0.004 (34.66)	44.58 ± 0.003 (44.08)	35.84 ± 0.002 (21.58)	64.49 ± 0.004 (21.29)	59.50 ± 0.006 (26.51)	41.40 ± 0.004 (31.90)	80.21 ± 0.006 (25.29)	78.21 ± 0.02 (23.51)	49.17 ± 0.05 (41.34)	115.48 ± 0.005 (24.00)	108.24 ± 0.004 (19.33)	79.57 ± 0.04 (25.41)
	KRA5	47.11 ± 0.002 (40.71)	45.40 ± 0.005 (41.48)	33.50 ± 0.003 (30.07)	59.17 ± 0.005 (32.21)	57.50 ± 0.003 (30.91)	37.20 ± 0.004 (46.78)	79.35 ± 0.003 (26.65)	75.48 ± 0.004 (27.98)	45.62 ± 0.02 (52.33)	108.55 ± 0.003 (31.91)	100.40 ± 0.005 (28.65)	78.50 ± 0.008 (27.13)
	SKD6	55.50 ± 0.004 (19.45)	52.49 ± 0.003 (22.36)	41.57 ± 0.005 (4.83)	63.06 ± 0.03 (24.06)	55.73 ± 0.77 (35.07)	48.34 ± 0.004 (12.96)	82.34 ± 0.002 (22.05)	72.78 ± 0.006 (32.72)	55.57 ± 0.008 (25.05)	117.50 ± 0.01 (21.87)	112.71 ± 0.37 (14.60)	73.42 ± 0.01 (35.93)
	NKM9	58.18 ± 0.009 (13.94)	55.70 ± 0.07 (15.31)	40.25 ± 0.01 (8.26)	64.50 ± 0.006 (21.28)	60.50 ± 0.006 (24.42)	46.50 ± 0.007 (17.41)	77.04 ± 0.06 (30.44)	74.66 ± 0.007 (29.38)	54.83 ± 0.007 (26.76)	105.66 ± 0.03 (35.53)	96.16 ± 0.02 (34.32)	88.93 ± 0.005 (12.22)
	Parental Mean	52.28 (26.80)	49.50 (29.76)	36.68 (18.79)	62.69 (24.78)	58.27 (29.19)	43.79 (24.69)	80.10 (25.47)	75.97 (27.15)	51.53 (34.86)	111.74 (28.15)	103.97 (24.24)	80.32 (24.24)

Table 4. Antioxidant properties of DPPH of cumin. Different superscript letters (a–f) indicate significant differences ($p < 0.05$) among genotypes mean under the same treatment, based on Duncan's multiple range test. The values in parentheses indicate the percentage superiority of the synthetic cultivar compared to each parental genotype.

Seed yield

Cumin seed yield is influenced by environmental conditions and genetic factors, and the results of this research showed that cumin seed yield decreased under the negative influence of drought stress (Table 2). Drought stress in plants leads to a reduction in leaf water content, resulting in stomatal closure and decreased photosynthesis, as well as affecting enzymatic activities and related processes. These factors contribute to flower drop, reduced seed weight, and ultimately a decrease in seed yield[27]. Similar reductions in cumin seed yield under drought conditions have been reported by Safari et al.[28] and Alinian and Razmjoo[29]. Other aromatic and medicinal crops have also shown similar results under drought stress conditions[30–32].

The use of polycross mating among parental genotypes to produce a synthetic variety played an effective role in compensating for the reduction in cumin seed yield under drought stress. Polycross mating, by creating a diverse genetic pool among parental genotypes and generating half-sib families, transferred superior characteristics to the progeny of the synthetic variety. Since an increase in cumin seed yield leads to enhanced productivity, economic benefits from exports, food security, and meeting market demands[32], it can be concluded that the increase in seed yield and the reduction in the negative impact of drought stress in the synthetic cumin variety not only confirms the validity of the selected breeding method for increasing yield under various environmental conditions but also indicates increased drought tolerance in the synthetic variety, which is very important for stable production.

Primary metabolites

Seed protein content

The seed protein content influenced by various factors, including plant species and the genetic content of the genotypes[33]. The synthetic cumin cultivar, with its superior genetic combination of the parental genotypes, possesses a higher protein content than its parents (Table 2).

Under drought stress condition, the protein content increased compared to normal condition, and water stress caused a higher increase in protein content in the synthetic cultivar (SYN2) (6.36%) compared to the average of the parental genotypes (5.08%). One of the major changes that occur in plants under drought stress is the alteration in the production of plant proteins, either through degradation or inhibition of the synthesis of some proteins, as well as the production of a small group of stress-specific proteins[34,35]. These changes in gene expression led to the activation or deactivation of enzymes and alterations in the specific structure of plant tissues. Drought stress also affects the status of polyribosomes involved in protein synthesis in tissues. Consequently, relative water deficiency during the flowering and seed filling stages results in an increased percentage of protein in seeds. Daneshian et al.[36] suggested that under drought stress conditions, with the reduction in seed size, proteins occupy a larger volume within the seed, which is a factor for the increased percentage of seed protein under drought stress. Similar observations of higher protein content under drought stress conditions have been reported in canola[33], wheat[37], and maize[38], which aligns with the results of this study. Therefore, it can be concluded that the increased production and development of seed proteins under drought stress in the synthetic cumin cultivar contribute to its improved drought tolerance.

Seed sugar content

Based on the results presented in Table 2, the sugar content of cumin seeds increased under drought stress irrigation conditions compared to normal conditions. Previous studies have reported varying changes in soluble sugar content under drought stress conditions, with some showing an increase[39] and others showing a decrease[40]. These variations depend on factors such as plant species, genotype, intensity, and duration of the stress, and the growth stage of the plant. Driesen et al.[41] suggested that during drought stress, stomata may close to prevent transpiration. However, stomatal closure affects gas exchange, leading to gradual reduction in carbon dioxide entry for photosynthesis and water loss through transpiration. Soluble sugars serve as an energy source for plant cells and play a crucial role in the plant's drought tolerance mechanism[42]. Also contributes to the elimination of reactive oxygen species (ROS) through the oxidative pentose phosphate pathway[43]. Therefore, increasing soluble sugar content through breeding techniques such as polycross hybridization is essential for enhancing drought tolerance. In this study, the synthetic cumin cultivar (SYN2) exhibited a 12.89% improvement in soluble sugar content compared to the average of the parental genotypes under drought stress condition. Since the accumulation of osmotic regulators like soluble sugars is associated with drought resistance[44], cultivating the synthetic cumin cultivar in arid and semi-arid regions is likely to result in high productivity.

Secondary metabolites

Percentage, yield, and composition of essential oil

Drought stress increased the content of cumin essential oil (Table 3). Drought stress is known to increase the essential oil content in medicinal plants and is considered a significant factor in enhancing the production of these valuable compounds[45]. Similar findings regarding the increase in cumin essential oil content under drought stress have been reported by other researchers[46,47]. However, the essential oil yield decreased under water shortage conditions due to a greater reduction in seed yield compared to the increase in essential oil content[48]. Other studies have also reported a significant reduction in essential oil yield under drought conditions[49,50]. The synthetic cultivar had the highest content and yield of essential oil in both normal and drought stress conditions. The essential oil content is primarily influenced by genetic factors[51]. Therefore, during the production of the synthetic variety, there is a high potential for transferring the desirable essential oil content trait to the progeny. In conclusion, the synthetic variety of cumin, with its high essential oil yield compared to other genotypes, holds commercial value and is recommended for use in the pharmaceutical, food, and cosmetic industries[52].

Monoterpene compounds, which are more than half of the constituents of cumin essential oil, play a crucial role in the aroma and flavor of plants and processed foods, making them highly valuable in industries such as

Condition		100 µg/ml			300 µg/ml			600 µg/ml			900 µg/ml		
		Ethanol	Methanol	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Water
Normal	SYN1	0.63	0.56	0.35	0.86	0.81	0.52	1.37	1.27	0.87	1.52	1.49	0.93
	SYN2	0.59 ± 0.008a	0.46 ± 0.006a	0.33 ± 0.004a	0.82 ± 0.003a	0.76 ± 0.001a	0.53 ± 0.0.07a	1.21 ± 0.007a	1.11 ± 0.001a	0.84 ± 0.004a	1.49 ± 0.003a	1.32 ± 0.005a	0.92 ± 0.003a
	YAR1	0.19 ± 0.15b (208.46)	0.08 ± 0.002d (514.34)	0.05 ± 0.005e (609.32)	0.24 ± 0.003f (238.74)	0.21 ± 0.005e (265.26)	0.19 ± 0.001d (183.92)	0.37 ± 0.002f (229.48)	0.32 ± 0.003f (243.54)	0.29 ± 0.003f (189.78)	0.58 ± 0.003f (157.17)	0.54 ± 0.002f (144.02)	0.49 ± 0.004e (87.45)
	GJA3	0.12 ± 0.005b (372.17)	0.11 ± 0.004 cd (332.45)	0.09 ± 0.003d (266.65)	0.29 ± 0.006d (187.64)	0.25 ± 0.003d (204.06)	0.22 ± 0.008 cd (142.90)	0.48 ± 0.004d (153.01)	0.47 ± 0.001d (137.99)	0.43 ± 0.0.004d (94.69)	0.66 ± 0.006d (125.29)	0.64 ± 0.001d (107.83)	0.58 ± 0.10c (58.10)
	KRA5	0.12 ± 0.001b (380.73)	0.11 ± 0.003 cd (337.43)	0.08 ± 0.003d (294.28)	0.27 ± 0.002e (207.74)	0.25 ± 0.002d (205.55)	0.24 ± 0.003c (124.05)	0.44 ± 0.007e (172.33)	0.39 ± 0.006e (183.46)	0.37 ± 0.003e (125.34)	0.61 ± 0.003e (142.77)	0.58 ± 0.002e (126.62)	0.56 ± 0.02d (64.00)
	SKD6	0.16 ± 0.003b (269.77)	0.13 ± 0.005c (251.35)	0.10 ± 0.002c (221.22)	0.36 ± 0.004c (131.19)	0.32 ± 0.006c (134.67)	0.30 ± 0.006b (77.57)	0.52 ± 0.002c (132.72)	0.49 ± 0.008c (127.91)	0.46 ± 0.001c (83.80)	0.69 ± 0.004c (116.72)	0.64 ± 0.005c (105.35)	0.57 ± 0.004c (59.97)
	NKM9	0.18 ± 0.006b (228.13)	0.18 ± 0.05b (159.79)	0.11 ± 0.001b (195.02)	0.38 ± 0.01b (115.37)	0.35 ± 0.001b (120.78)	0.32 ± 0.001b (65.89)	0.63 ± 0.002b (93.24)	0.57 ± 0.001b (95.28)	0.54 ± 0.002b (55.10)	0.79 ± 0.01b (89.07)	0.75 ± 0.005b (76.28)	0.70 ± 0.002b (30.64)
	Parental Mean	0.16 (279.02)	0.12 (286.95)	0.09 (279.47)	0.31 (168.14)	0.28 (176.39)	0.25 (110.42)	0.49 (148.37)	0.45 (148.15)	0.42 (100.70)	0.67 (123.72)	0.63 (109.51)	0.58 (57.91)
Stress	SYN1	–	–	–	–	–	–	–	–	–	–	–	–
	SYN2	0.61 ± 0.002	0.50 ± 0.002	0.47 ± 0.007	1.06 ± 0.002	0.78 ± 0.01	0.52 ± 0.007	1.42 ± 0.007	1.30 ± 0.04	0.89 ± 0.02	1.72 ± 0.005	1.47 ± 0.03	0.95 ± 0.001
	YAR1	0.13 ± 0.005 (373.91)	0.10 ± 0.003 (387.10)	0.07 ± 0.005 (554.61)	0.26 ± 0.005 (301.86)	0.32 ± 0.002 (141.47)	0.24 ± 0.002 (113.56)	0.38 ± 0.002 (268.91)	0.47 ± 0.004 (178.26)	0.32 ± 0.001 (173.81)	0.59 ± 0.005 (193.36)	0.69 ± 0.005 (112.56)	0.52 ± 0.001 (84.40)
	GJA3	0.14 ± 0.005 (327.73)	0.12 ± 0.005 (305.26)	0.09 ± 0.001 (411.58)	0.35 ± 0.001 (202.96)	0.32 ± 0.005 (143.55)	0.25 ± 0.006 (111.25)	0.53 ± 0.005 (166.00)	0.52 ± 0.007 (151.18)	0.46 ± 0.004 (94.48)	0.68 ± 0.002 (153.49)	0.66 ± 0.001 (122.18)	0.62 ± 0.003 (52.86)
	KRA5	0.15 ± 0.003 (300.47)	0.12 ± 0.005 (322.33)	0.09 ± 0.004 (406.12)	0.30 ± 0.002 (258.33)	0.26 ± 0.001 (202.38)	0.26 ± 0.004 (97.75)	0.43 ± 0.004 (229.50)	0.42 ± 0.006 (212.97)	0.41 ± 0.003 (116.05)	0.68 ± 0.004 (154.53)	0.62 ± 0.004 (135.94)	0.59 ± 0.002 (61.64)
	SKD6	0.20 ± 0.002 (205.72)	0.19 ± 0.005 (170.41)	0.13 ± 0.002 (275.46)	0.40 ± 0.005 (162.41)	0.39 ± 0.001 (102.60)	0.32 ± 0.004 (64.83)	0.58 ± 0.004 (146.68)	0.56 ± 0.005 (133.43)	0.52 ± 0.002 (68.89)	0.73 ± 0.001 (137.45)	0.69 ± 0.004 (113.08)	0.59 ± 0.008 (60.32)
	NKM9	0.24 ± 0.004 (154.16)	0.21 ± 0.005 (134.31)	0.15 ± 0.003 (220.17)	0.46 ± 0.002 (131.32)	0.41 ± 0.003 (93.44)	0.37 ± 0.009 (42.37)	0.69 ± 0.004 (104.29)	0.66 ± 0.005 (96.05)	0.58 ± 0.001 (53.17)	0.85 ± 0.005 (101.68)	0.82 ± 0.003 (79.60)	0.75 ± 0.003 (26.71)
	Parental Mean	0.17 (253.34)	0.15 (236.60)	0.11 (344.65)	0.35 (199.15)	0.34 (130.84)	0.29 (81.40)	0.52 (170.94)	0.52 (148.08)	0.46 (93.26)	0.70 (144.47)	0.70 (110.93)	0.62 (54.89)

Table 5. Antioxidant properties of reducing power of cumin. Different superscript letters (a–f) indicate significant differences ($p < 0.05$) among genotypes mean under the same treatment, based on Duncan's multiple range test. The values in parentheses indicate the percentage superiority of the synthetic cultivar compared to each parental genotype.

Fig. 1. Comparison of physical characteristics of seeds in parental genotypes and synthetic varieties under normal irrigation and drought stress conditions (N and S indicate normal irrigation condition and drought stress, respectively).

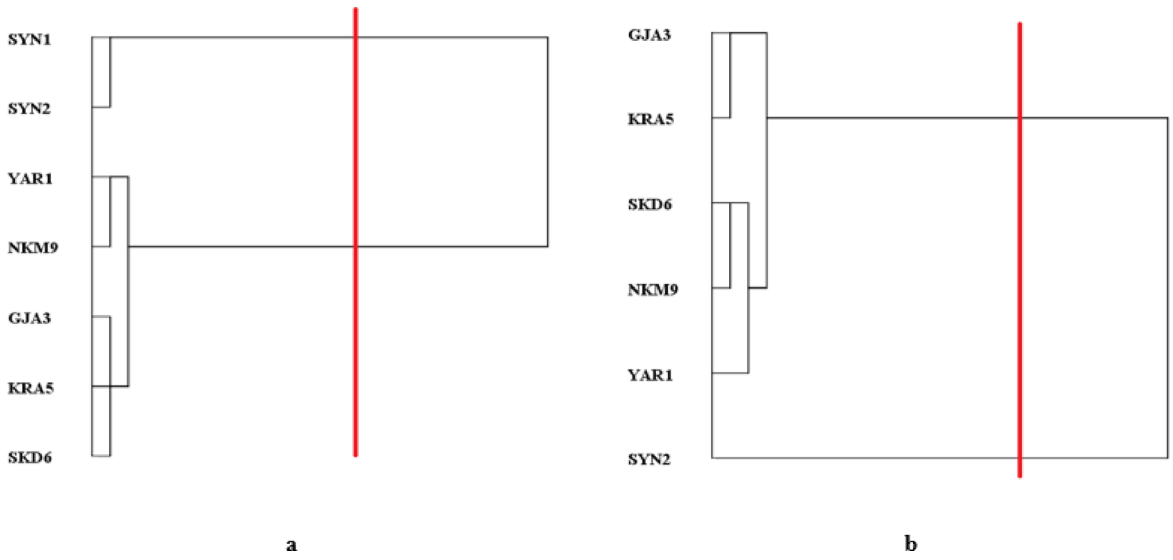
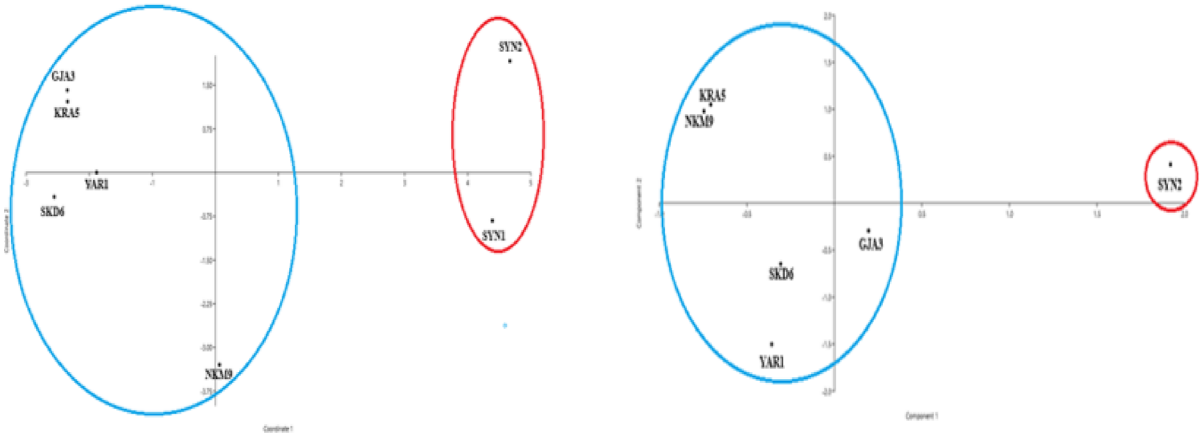


Fig. 2. Dendrogram of parental genotypes and the cumin synthetic variety, constructed using the Ward method and Euclidean distance, under two irrigation conditions: (**a**) normal, and (**b**) drought stress.

cosmetics, pharmaceuticals, and food[47]. The biosynthesis pathway of these monoterpenes under water stress conditions involves key genes such as limonene synthase and flavone synthase[47]. Therefore, the increase of gamma-terpinene and aldehyde compounds during drought stress can also indicate an increase in tolerance to drought stress. Also, previous studies have reported the range of Cumin aldehyde content in cumin to be between 27% and 50% [19,53]. In this study, by utilizing the rich genetic resources of the parental genotypes, it was



a b

Fig. 3. Principle component analysis of the parental genotypes and cumin synthetic variety under two conditions: (**a**) normal irrigation and (**b**) drought stress.

Fatty acids (%)	Normal							Stress						
	SYN1	SYN2	YAR1	GJA3	KRA5	SKD6	NKM9	SYN1	SYN2	YAR1	GJA3	KRA5	SKD6	NKM9
Oil Content (%)	–	8.78	5.30	4.30	5.16	4.44	8.22	–	12.04	8.10	4.60	6.82	7.68	9.10
Palmitic acid (C16:0)	4.16	5.03	6.19	4.49	6.80	4.85	9.41	–	5.82	5.03	6.04	6.83	5.88	6.72
Stearic acid (C18:0)	1.09	0.99	1.33	0.75	1.84	0.84	1.63	–	1.04	0.81	1.32	1.80	1.26	1.18
Oleic acid (C18:1)	61.49	60.46	58.64	60.03	53.08	59.28	53.63	–	61.65	61.86	56.21	53.20	56.70	58.88
Linoleic acid (C18:2)	30.91	30.99	29.91	31.52	28.67	30.60	30.20	–	29.29	29.92	30.10	28.92	28.64	30.62
Alpha-linolenic acid (C18:3)	0.73	0.65	0.74	0.51	0.85	0.64	1.22	–	0.44	0.36	0.80	1.00	0.89	0.68
ΣSFA	5.25	6.02	7.52	5.24	8.64	5.70	11.03	–	6.85	5.84	7.36	8.63	7.14	7.90
ΣMUFA	61.49	60.46	58.64	60.03	53.08	59.28	53.63	–	61.65	61.86	56.21	53.20	56.70	58.88
ΣPUFA	31.64	31.64	30.66	32.03	29.52	31.24	31.42	–	29.73	30.28	30.91	29.92	29.53	31.31
ΣUFA	93.13	92.10	89.29	92.06	82.61	90.51	85.05	–	91.38	92.14	87.12	83.12	86.23	90.18
PUFA/SFA	0.50	0.51	0.51	0.53	0.54	0.52	0.56	–	0.48	0.48	0.54	0.54	0.51	0.52

Table 6. Effect of drought on fatty acid composition of *Cuminum cyminum* L. seeds.

possible to enhance the essential oil content and increase the amount of Cumin aldehyde in the synthetic variety of cumin.

Polyphenolic compounds

Increased content of cumin polyphenol compounds in the conditions of drought stress was compliant with the findings of other researchers[46,54] (Table 2). The increase in polyphenolic compounds is a response to oxidative stress caused by the formation of reactive oxygen species under drought conditions. These compounds help stabilize cell membranes and prevent lipid peroxidation[8,55]. The greater increase in polyphenolic content in the synthetic variety suggests that it possesses stronger antioxidant activities, enabling it to counteract reactive oxygen species more effectively[56].

It is important to note that the levels of polyphenols in different extracts can vary depending on the sample preparation methods and extraction techniques. In this study, ethanol extracts exhibited the highest amounts of polyphenolic compounds under both normal irrigation and water stress conditions, indicating the superior extraction power of ethanol compared to methanol and water solvents. The extraction of polyphenolic compounds from plant materials is influenced by the polarity of the solvents used and the solubility of these compounds in different solvents[57]. On the other hand, the biosynthesis of polyphenolic compounds is largely influenced by genetic factors, and previous studies have confirmed the impact of genetics on the production of secondary metabolites in cumin[58]. Therefore, the increased metabolic content in the synthetic variety of cumin can be attributed to the utilization of heterosis. However, the solvent used for extraction should also be

considered, as its polarity can affect the polyphenolic content of the extract[59], which aligns with the findings of this study.

Overall, the increase in polyphenolic compounds indicates an enhanced ability to scavenge reactive oxygen species and, consequently, an increase in drought tolerance. These compounds are significantly influenced by water scarcity[60]. Therefore, the production of a drought-tolerant synthetic variety of cumin through polycross breeding techniques can lead to a substantial increase in metabolic content, thereby enhancing the nutritional and commercial value of this plant and emphasizing the importance of increased production.

Antioxidant properties

Higher increase in the antioxidant properties of the extract with increasing concentration of extract can be attributed to the higher concentration of phenolic compounds, which provide more hydroxyl groups for the donation of hydrogen to free radicals and thus increase the inhibitory power of the extract. These findings are consistent with previous studies that have reported a positive correlation between antioxidant activity and polyphenolic content in C. sativum and Cakile maritime[61,62].

The content of cumin polyphenolic compounds increased in irrigation conditions of drought stress compared to normal conditions (Tables 4 and 5). During drought stress, plants employ antioxidant defense systems to protect against reactive oxygen species (ROS) in various cellular components such as chloroplasts, mitochondria, and peroxisomes. The antioxidant capacity of a plant plays a crucial role in its resistance to different stresses, and increasing the levels of antioxidants can help prevent damage[63]. Phenols, flavonols, and flavonoids, which are low molecular weight antioxidants, work in coordination to effectively neutralize harmful radicals[64]. Therefore, based on the findings of this research, it can be concluded that polycross breeding has the potential to genetically enhance the antioxidant properties of the synthetic variety, making it a promising approach for the production of drought-tolerant plants.

Physical seed traits

The reduction in physical traits under drought stress is a commonly observed phenomenon in plants[65] which also corresponds to the findings of this study. Drought stress significantly hampers germination and seedling vigor, resulting in a decrease in seed size and yield[66]. Studies have indicated that plants tend to expedite the cessation of initial flower growth under water scarcity, leading to the production of smaller seeds[67]. However, the synthetic variety exhibited the highest values for thousand-seed weight, area, perimeter, length, and width under both irrigation conditions, demonstrating its superior resilience and greater tolerance to drought compared to the parental genotypes (Fig. 1). Also, the thousand-seed weight trait due to the lower effect of drought stress than other physical traits can be considered as a physical indicator of drought tolerance.

Cluster and principal component (PCA) analysis

The results of cluster and principal component (PCA) analysis showed that, in comparison to the parental genotypes, the synthetic variety of cumin exhibited superior traits, notably higher seed yield, metabolite content, and better antioxidant properties. This advantage of the synthetic variety over its parental genotypes is often linked to genetic recombination, which fosters greater genetic diversity and enhances adaptation to environmental fluctuations. The process of genetic recombination not only boosts heterosis but also maintains genetic variability within key genomic regions, allowing for the reshuffling of genetic material and the formation of new allelic combinations. These newly combined alleles contribute to the expression of improved traits in the synthetic variety, a result that aligns with the findings of this study.

Percentage and composition of fatty acids

Increasing the content of cumin oil under drought stress conditions can be attributed to the reduction in seed size, which allows oil and protein to occupy a larger proportion of the seed space, thereby leading to an elevation in the oil percentage during drought stress conditions[36]. Also, the higher seed oil content of cumin synthetic cultivar in both normal irrigation conditions and drought stress can be attributed to the synthetic variety's superior genetic control over the oil percentage. However, the synthetic variety experienced a more pronounced reduction in unsaturated fatty acids under drought stress compared to normal irrigation conditions, in contrast to the parental genotypes (Table 6). Similar findings have been reported in studies on sunflower[68], camelina[69], and wheat[37], which have all observed a decrease in unsaturated fatty acids under drought stress conditions. This outcome underscores the impact of water deficiency on the unsaturation level of fatty acids, thereby influencing the quality and stability of cumin oil.

Materials and methods

Plant materials and growth conditions

The synthetic variety of cumin was developed through a single polycross breeding event involving the random mating of five superior genotypes (YAR1, GJA3, KRA5, SKD6, and NKM9). Seeds of these genotypes were obtained from the cumin gene bank of the Faculty of Agricultural Technology (Aborihan), University of Tehran, Iran. These genotypes were selected for their high general combining ability in traits associated with seed yield and end-of-season drought tolerance, as evidenced by prior studies[28,70,71]. Polycross breeding was chosen due to its efficacy in generating a broad genetic base through random mating, which is particularly advantageous for open-pollinating species like cumin, enhancing the potential to combine multiple desirable traits such as drought tolerance and metabolite content. The evaluation of the first (SYN1) and second (SYN2) synthetic generations, alongside the parental genotypes, was conducted over two growing seasons (2021–2022) at two distinct geographical locations: Pakdasht, Tehran, Iran (latitude 35 degrees 29 min north, longitude 51 degrees 40 min east, and altitude 1027 m above sea level), and Bidgol, Kashan, Isfahan, Iran (latitude 34 degrees 05 min

north, longitude 51 degrees 43 min east, and altitude 987 m above sea level). While the polycross was performed as a single event, the multi-environment testing across two years and locations provided a robust framework to assess the consistency of the synthetic variety's performance, partially compensating for the lack of repeated polycross events. The experiment was designed in a randomized complete block design with three replications. The selected geographical areas are categorized as arid regions with minimal rainfall from early April to mid-October. After the completion of the vegetative stage and initiation of the reproductive phase (onset of flowering), the plants were subjected to two different irrigation regimes: 100% field capacity (normal irrigation) and 30% field capacity (drought stress)[72]. The seed yield after complete ripening was calculated as grams per square meter, taking into account the optimal plant density (120 plants per square meter), and seeds were air-dried and stored at 4 degrees Celsius for further analysis.

Evaluation of primary metabolites

Seed protein content

The seed protein content was estimated using the Kjeldahl method described in AOAC[73]. This method involves protein digestion, distillation, and nitrogen determination by titration. The seed protein content was calculated as a percentage by multiplying the nitrogen content by a factor of 6.25.

Seed carbohydrate content

The total carbohydrate content of seeds was measured using the method described by McCready et al.[74]. For total carbohydrate measurement, 200 µl of concentrated ethanolic extract mixed with 3 milliliters of anthrone were heated for 20 min in a water bath at 100 degrees Celsius. The absorbance of each sample was measured at a wavelength of 620 nanometers after cooling.

Evaluation of secondary metabolites

Percentage, yield, and composition of essential oil

The essential oil was extracted from seeds using the water distillation method and a Clevenger apparatus. The percentage of essential oil was obtained according to the European pharmacopoeia[75]. The essential oil yield was calculated based on the product of the percentage of essential oil and the yield of dry matter per unit area.

Identification of essential oil compounds was performed by gas chromatography (Agilent 7890 A) coupled with mass spectrometry (Agilent 5975 C) using a HP Innowax Capillary column (60.0 m × 0.25 mm × 0.25 µm). Essential oils were diluted 1:9 with hexane. GC-MS/FID analysis was performed with a split ratio of 40:1. The injection volume and temperature were 1 µl and 250 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 0.8 milliliters per minute. The temperature program was set as follows: 60 °C for 10 min, followed by an increase of 4 °C per minute up to 250 °C, which was maintained for 10 min. The mass spectrum was monitored from 35 to 450 amu, and the electron impact ionization mode at 70 eV was used. Relative percentages of compound compositions were calculated based on peak areas in GC-FID chromatograms, and the compounds were identified using Wiley 7 and Oil Adams libraries[76].

Extraction and measurement of polyphenols

Extraction of extracts

For extraction of seed extracts, 2.5 g of air-dried seed powder were mixed with 25 milliliters of solvent for 30 min. After 24 h, the extracts were filtered through Whatman filter paper No. 4 and dried under vacuum and stored at 4 degrees Celsius until further analysis. Seed extracts were obtained using different solvents, including 80% ethanol, 80% methanol, and water.

Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent method[77]. Plant extracts were mixed with 2.0 mM Folin-Ciocalteu reagent and incubated for 5 min. The reaction was neutralized with saturated sodium carbonate solution (2 milliliters, 75 g per liter) and incubated for 90 min at room temperature. The bsorbance of the reaction mixture was measured at a wavelength of 760 nanometers using a spectrophotometer. Gallic acid was used as the standard, and the results were expressed as milligrams of gallic acid equivalents per gram of seed extract.

Total flavonoid content

For determination of total flavonoid content, plant extract was mixed with 0.3 milliliters of 5% sodium nitrite solution (V/V). The reaction mixture was allowed to stand for 5 min at room temperature, then 0.3 milliliters of aluminum chloride and 2 milliliters of 1 M sodium hydroxide were added. Finally, the reaction mixture was diluted, and absorbance was measured at 510 nanometers. The standard curve of quercetin was used for calculation, and the results were expressed as milligrams of quercetin equivalent per 100 milligrams of extract[78].

Total flavonol content

The total flavonol content was determined based on aluminum chloride colorimetry. For this purpose, 1 milliliter of plant extract was mixed with 2 milliliters of 2% aluminum chloride, 6 milliliters of 5% sodium acetate, and 1 milliliter of extract solvent. After 2.5 h at room temperature the absorbance of the reaction mixture was measured at 445 nanometers. The standard curve of quercetin was used for calculation, and the results were expressed as milligrams of quercetin equivalent per 100 milligrams of extract[79].

Evaluation of antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to the method described by Hanato et al.[80]. Plant extract at various concentrations (25, 50, 90, and 130 micrograms per milliliter) was added to 0.2 mM DPPH methanolic solution. After shaking, the mixture was incubated for 30 min in the dark at room temperature, and absorbance was measured at 517 nanometers. BHA was used as a positive control, and methanol was used as a negative control. The percentage inhibition of DPPH radical was calculated using the formula: [(A_blank - A_sample) / A_blank] × 100%.

Reducing power

To evaluate the reducing power, 1 milliliter of plant extract at various concentrations (100, 300, 600, and 900 micrograms per milliliter) was mixed with 2.5 milliliters of 0.2 M phosphate buffer (pH 6.6) and 2.5 milliliters of 1% potassium ferricyanide. The reaction mixture was incubated at 50 degrees Celsius for 20 min, then 2.5 milliliters of 10% trichloroacetic acid were added, followed by centrifugation at 3000 rpm for 10 min. Finally, the supernatant was mixed with distilled water and 0.1% ferric chloride solution. Absorbance was measured at 700 nanometers, and ascorbic acid was used as a positive control[80].

Assessment of seed physical traits

Ten seeds of each parental genotype and synthetic variety (SYN2) of cumin were randomly selected and their length, width, area, and perimeter were examined using Digimizer software. Additionally, the weight of 1000 seeds (grams) was determined by randomly weighing 100 seeds with an accuracy of 0.01 g and multiplying by 10.

Content and composition of fatty acids

Three 5-gram samples of seeds were ground into powder using an electric mill. Oil content was extracted using a Soxhlet apparatus with n-hexane solvent for 6 h. After extraction, the solvent was separated from the oil using a rotary evaporator at 60 degrees Celsius and 500 mbar pressure for one hour. The oil yield was calculated based on the weight of dry matter (grams of oil per gram of dry matter)[81].

Identification of seed fatty acid composition was performed using a gas chromatography apparatus (Agilent model 6890, UK) equipped with flame ionization detectors (FID) and a DB-WAX column (30 m in length, 0.25 mm internal diameter, and 0.25 micrometer film thickness) made of fused silica with a polar phase. Hydrogen gas was used as the carrier gas. After conversion of fatty acids to methyl esters (FAME), samples were injected into the gas chromatograph under the following conditions: hydrogen gas flow rate of 1 milliliter per minute, column temperature of 180 degrees Celsius, injector temperature of 250 degrees Celsius, detector temperature of 260 degrees Celsius, and injection volume of 20 µl. The retention time of each fatty acid was compared with the retention time of the corresponding standards under the same experimental conditions, and the percentage of each fatty acid was determined[82]. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were calculated by summing up the percentages of relevant fatty acids.

Data analysis

All measurements were performed in triplicate, and the results were expressed as the mean. Before analysis of variance (ANOVA), the Kolmogorov-Smirnov test was performed to check the normal distribution of errors. The Bartlett test was used to test the homogeneity of residual variances. Furthermore, the data were analyzed statistically using ANOVA with the SPSS program. The significance of differences between means was determined using Duncan's multiple range test ($p < 0.05$). Principal component analysis (PCA) and clustering were performed using PAST and SPSS version 26 software, respectively.

Conclusion

The management of cumin production under drought stress conditions is of utmost importance for maximizing seed yield and enhancing the production of its active compounds. The breeding method employed to develop the synthetic variety of cumin offers a promising approach to introduce genetic recombination through heterosis, thereby enabling the plant to better cope with drought stress. The results of this study clearly indicate that the synthetic variety outperforms its parental genotypes in all studied traits, irrespective of whether the plants are subjected to normal irrigation or drought stress conditions. Consequently, the production of the synthetic variety emerges as a suitable and effective breeding method for enhancing seed yield, metabolite content, antioxidant properties, and drought tolerance in cumin. This is achieved through the utilization of polycrosses between superior parental genotypes, facilitating the transfer of desirable traits to the progeny for genetic improvement of cumin. This highlights the significance of producing the synthetic variety in medicinal plant production to ensure increased productivity and quality under challenging environmental conditions.

Data availability

All data generated or analyzed during this study are included in the article. There are no supplementary files associated with this study.

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Author contributions
S.M.M.M. is the supervisor and suggested the research idea, M.A.B. and S.M.M.M. designed the experiments, collected data, and contributed to interpreting the results. M.A.B. wrote the paper and S.M.M.M. edited the original draft. All authors approved the final manuscript to be published.

Declarations

Competing interests
The authors declare no competing interests.

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Not required as no human data or animal samples were used.

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All authors agreed to participate in the present work.

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All the authors agreed with the present publication.

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