

Yield and Physiological Responses of Chickpea Cultivars to Arbuscular Mycorrhizal Fungi Inoculation under Drought Stress

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Abstract

Terminal drought is a principal constraint on chickpea (*Cicer arietinum* L.) productivity in rainfed tracts, where the crop matures on progressively receding residual soil moisture. A field investigation was conducted during the Rabi seasons of 2022-23 and 2023-24 at Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra, India, to evaluate the yield and physio-biochemical responses of seven chickpea genotypes (PDKV Kanak, PDKV Kanchan, PKV Kabuli-2, PKV Kabuli-4, JAKI-9218, Chandrapur Chanoli and Gulak-1) to moisture stress following inoculation with vesicular arbuscular mycorrhiza (VAM). The experiment followed a Factorial Randomized Block Design with two water regimes (irrigated and moisture stress) and seven varieties. Moisture stress significantly reduced seed yield (by 10.3–20.2%), straw yield (by 9.8–18.0%), total chlorophyll content at 90 days after sowing (DAS; by 13.8–20.2%) and relative water content at 90 DAS (by 15.3–25.7%), while significantly increasing proline accumulation (by 132.7–189.9% at pod filling) and superoxide dismutase (SOD) activity (by 31.9–86.7%), relative to irrigated conditions, in the pooled analysis of both seasons. JAKI-9218 consistently showed the smallest relative decline in yield, chlorophyll content and relative water content, together with the greatest proline accumulation and SOD induction under stress, identifying it as the most drought-tolerant genotype, followed by PDKV Kanak and PDKV Kanchan; Gulak-1 was the most drought-susceptible. These results indicate that VAM inoculation, combined with inherently tolerant genotypes such as JAKI-9218, can help sustain chickpea productivity under terminal drought through improved water relations, retained photosynthetic pigments, and enhanced osmotic and antioxidative adjustment, offering promising material for drought-tolerance breeding in chickpea.

1. Introduction

Chickpea (*Cicer arietinum* L.) is the second most important food legume cultivated worldwide, particularly in developing countries, and is an economically valuable, protein-rich crop (Varshney et al., 2013; Vishnu et al., 2018; Singh et al., 2023). Globally, chickpea occupies 18.86 million hectares with a production of 15.08 million tonnes and an average productivity of 1.01 t ha⁻¹ (FAOSTAT, 2023). During 2021-22 (fourth advance estimate), India produced 13.75 million tonnes of chickpea from an area of 10.91 million ha, with a productivity of 12.6 q ha⁻¹ (DES, 2023), contributing about 70% of world production and making India the largest producer (Wallace et al., 2016; Kushwah et al., 2021).

Chickpea production is constrained by several abiotic stresses, of which drought, and low and high temperatures are the most important (Ryan, 1997; Jha et al., 2014). Being a dry, cool-season crop, chickpea is largely grown rainfed on residual soil moisture after the monsoon season (Rizvi et al., 2014; Niveditha et al., 2022), and progressively receding soil moisture towards the end of the crop season (terminal drought) often causes heavy yield losses (Gaur et al., 2013; Kumar et al., 2018; Asati et al., 2024). About 90% of the chickpea crop is grown under rainfed conditions without any supplemental irrigation, so that both the vegetative and reproductive phases are adversely affected by water deficit (Maqbool et al., 2017; Yimer et al., 2024), with the crop being most sensitive to water stress at the pre-flowering and early pod-filling stages (Tiwari et al., 2023). Terminal drought is consequently regarded as

one of the most important constraints on yield potential, capable of reducing chickpea yield by 50–70% (Kaloki et al., 2019; Istanbuli et al., 2025), and its impact is expected to intensify as unpredictable climate change increases the frequency of drought and temperature extremes, adversely affecting plant development, physiology and grain yield (Rani et al., 2020; Karkee et al., 2026; Zargar et al., 2026).

Drought adversely affects plant nutrition and key physiological processes such as relative water content and photosynthetic rate (Jabborova et al., 2022; Jerbi et al., 2022; Sachdeva et al., 2022) through its effects on morphological, biochemical and molecular mechanisms in plants (Nader et al., 2024), and alters both primary and secondary metabolism in plant tissues (Itam et al., 2020). Water stress is well known to induce oxidative damage in plant cells through the overproduction of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$) (Sachdeva et al., 2022; Sharma et al., 2012), which seriously restrict normal growth, development, survival and productivity.

Vesicular arbuscular mycorrhizal (VAM) fungi play an important role in enhancing plant resilience to drought stress through their symbiotic association with plant roots (Boutasknit et al., 2020). VAM fungi improve nutrient uptake – particularly of phosphorus – enhance water absorption, and strengthen plant defence mechanisms against abiotic stress by facilitating a better root architecture and improved physiological efficiency (Berruti et al., 2014; Chandrasekaran, 2024). VAM inoculation therefore has the potential to mitigate the adverse effects of moisture stress and to sustain chickpea productivity in drought-prone regions, and is increasingly regarded as an agroecological solution that benefits crops under both stressful and non-stressful conditions (Ferioun et al., 2025).

Because drought tolerance is a complex, low-heritability trait, morphological and physiological parameters that are closely linked to it need to be identified so that superior, drought-tolerant genotypes can be selected and bred more effectively. Evaluation and selection of available chickpea varieties on the basis of their morpho-physiological and biochemical responses under drought, together with the use of bio-inoculants such as VAM, are therefore essential steps towards improving the drought tolerance of the crop. Comparative information on how a range of chickpea genotypes, in combination with VAM inoculation, respond to terminal drought under the Vertisol-semi-arid conditions of central India remains limited. The present investigation was accordingly undertaken with the following objectives: (i) to evaluate the effect of terminal (moisture-stress) drought on seed and straw yield of seven chickpea genotypes grown with VAM inoculation; (ii) to assess VAM-mediated changes in key physiological and biochemical indicators of drought stress – chlorophyll content, relative water content, proline accumulation and superoxide dismutase activity – under irrigated and moisture-stress conditions; and (iii) to identify and rank genotypes for comparative drought tolerance for use in future chickpea breeding and cultivation programmes.

2. Materials and Methods

2.1 Experimental site

A field investigation was conducted over two Rabi seasons (2022-23 and 2023-24) at the experimental farm of Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra, India, situated at 20°42' N latitude and 77°00' E longitude at an elevation of approximately 286 m above mean sea level. The site falls in the Western Vidarbha agro-climatic zone of Maharashtra, within the Western Plateau and Hills agro-climatic region of India, and has a broadly semi-arid climate. The soil of the experimental site is a Vertisol of moderate fertility with good moisture-retention capacity.

2.2 Experimental design and treatments

The experiment was laid out in a Factorial Randomized Block Design (FRBD) with two factors: Factor A comprised two water-regime conditions (irrigated and moisture stress) and Factor B comprised seven chickpea genotypes – PDKV Kanak, PDKV Kanchan, PKV Kabuli-2, PKV Kabuli-4, JAKI-9218, Chandrapur Chanoli and Gulak-1 – replicated across blocks. The water regime was fixed on the basis of the number of irrigations given: a common palco (pre-sowing) irrigation was applied under both conditions, after which the irrigated treatment received two further irrigations at the critical growth stages of flower initiation (40–45 DAS) and pod filling (65–70 DAS), while the moisture-stress treatment received no further irrigation, exposing the crop to progressive terminal drought. To enhance stress resilience and nutrient uptake, vesicular arbuscular mycorrhiza (VAM) was applied at sowing under the moisture-stress condition, both as a seed treatment (3 g kg⁻¹ seed) and as a soil application (12 g plot⁻¹) (Havargi, 2007).

2.3 Physiological and biochemical determinations

Total chlorophyll content (mg g⁻¹ fresh weight)

Chlorophyll a, chlorophyll b and total chlorophyll content were estimated from chickpea leaves at the pod-formation stage. Leaf tissue (0.25 g), cut into pieces after removing the veins, was macerated in a pestle and mortar with 80% acetone and made up to 25 mL with 80% acetone in a volumetric flask. Optical density was recorded on a spectrophotometer at 645, 652 and 663 nm, and chlorophyll a, chlorophyll b and total chlorophyll content were calculated using the equations of Sadasivam and Manickam (1996):

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = [12.7(A_{663}) - 2.69(A_{645})] \times V / (1000 \times W)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = [22.9(A_{645}) - 4.68(A_{663})] \times V / (1000 \times W)$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = [20.2(A_{645}) + 8.02(A_{663})] \times V / (1000 \times W)$$

where A = absorbance at the given wavelength, V = final volume of extract (mL), and W = fresh weight of the leaf sample (g).

Relative water content, RWC (%)

The third fully expanded leaf from the top was sampled at mid-day, packed immediately in humidified polythene bags, transported to the laboratory on ice, and weighed to obtain the fresh weight. Leaves

were then floated on distilled water in a Petri dish for 3 h to attain full turgidity and re-weighed to obtain the turgid weight, after which they were oven-dried at 65°C for 72 h to a constant dry weight. RWC was calculated following Barrs and Weatherley (1962):

$$\text{RWC (\%)} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100$$

Proline content ($\mu\text{mol g}^{-1}$ fresh weight)

Proline was estimated spectrophotometrically at 70 days after sowing by the ninhydrin method of Bates et al. (1973). A 0.25 g fresh leaf sample was homogenized in 3.0 mL of 3% sulfosalicylic acid and centrifuged at 1000 rpm for 15 min; 2.0 mL of the supernatant was reacted with 2.0 mL of acid-ninhydrin reagent (ninhydrin in glacial acetic acid), heated at 100°C for 60 min, and the reaction was terminated in an ice bath. The chromophore was extracted with 4.0 mL of toluene, and the absorbance of the pink toluene layer was read at 520 nm. Proline concentration was determined from a standard curve prepared with D-proline.

Superoxide dismutase (SOD) activity (U mg^{-1} protein)

Crushed leaf tissue (25 mg) was extracted in 250 μL of 0.1% trichloroacetic acid and centrifuged at 10,000 rpm for 20 min. To 160 μL of the supernatant, 160 μL of phosphate buffer and 680 μL of 1 M potassium iodide were added, and after 1 h in the dark the absorbance of the reaction mixture was recorded at 390 nm as a measure of SOD activity.

2.4 Statistical analysis

Data recorded for each character were subjected to analysis of variance (ANOVA) appropriate to the Factorial Randomized Block Design, separately for Rabi 2022-23 and 2023-24, and as a pooled analysis over the two seasons. Treatment means were compared using the Critical Difference (CD) at the 5% level of probability; differences among means smaller than the relevant CD were considered statistically at par (non-significant). Standard errors of the mean ($\text{SEm} \pm$) and CD (5%) are reported alongside treatment means in each table.

3. Results and Discussion

3.1 Seed yield and straw yield

Different water-regime conditions had a significant influence on seed yield (Table 1). Significantly higher seed yield (1632.71, 1671.71 and 1652.21 kg ha^{-1}) was recorded under irrigated conditions compared with moisture stress (1407.29, 1415.29 and 1411.29 kg ha^{-1}) during Rabi 2022-23, 2023-24 and in the pooled analysis, respectively. Significant differences in seed yield were also observed among varieties in both years and in the pooled analysis: PDKV Kanak (1837.25 kg ha^{-1} , pooled) recorded the significantly highest seed yield, statistically at par with PDKV Kanchan (1767.00 kg ha^{-1}) and JAKI-9218 (1725.75 kg ha^{-1}).

ha⁻¹), while the lowest seed yield was recorded in PKV Kabuli-4 (1128.50 kg ha⁻¹), on par with PKV Kabuli-2 (1201.50 kg ha⁻¹).

The water regime × variety interaction was significant for seed yield in both seasons and in the pooled analysis (Table 3). Under irrigated conditions, PDKV Kanak (1961.50 kg ha⁻¹, pooled) gave the significantly highest seed yield and PKV Kabuli-4 (1226.00 kg ha⁻¹) the lowest, while under moisture stress, PDKV Kanak again recorded the highest seed yield (1713.00 kg ha⁻¹), statistically at par with PDKV Kanchan (1640.00 kg ha⁻¹) and JAKI-9218 (1632.00 kg ha⁻¹); PKV Kabuli-4 again recorded the lowest (1031.00 kg ha⁻¹). Expressed as a percentage reduction from the irrigated to the moisture-stress condition, JAKI-9218 showed the smallest decline in seed yield (10.3%), followed by PDKV Kanak (12.7%) and PDKV Kanchan (13.4%), while Gulak-1 showed the largest reduction (20.2%) (Fig. 1a), indicating that JAKI-9218 was the most moisture-stress-tolerant genotype for seed yield, followed by PDKV Kanak and PDKV Kanchan, while Gulak-1 was the most susceptible.

A closely similar pattern was evident for straw yield (Tables 1 and 3; Fig. 1b): irrigated conditions produced significantly higher straw yield (2097.21 kg ha⁻¹, pooled) than moisture stress (1826.79 kg ha⁻¹), PDKV Kanak (2323.00 kg ha⁻¹) recorded the highest straw yield among varieties (at par with PDKV Kanchan and JAKI-9218), and the water regime × variety interaction was significant, with JAKI-9218 showing the smallest reduction under moisture stress (9.8%), followed by PDKV Kanak (10.4%) and PDKV Kanchan (10.7%), and Gulak-1 the largest reduction (18.0%).

The higher vegetative and reproductive expression under irrigated conditions indicates that additional irrigation at flower initiation and pod filling allowed the crop to more fully express its inherent yield potential. Under moisture-stressed conditions, VAM inoculation is likely to have helped plants access more water and nutrients, thereby mitigating — though not eliminating — the yield loss caused by terminal drought; the yield decline under stress, although significant, was consequently less pronounced than it would otherwise be. Similar beneficial effects of adequate moisture and of biological inputs on chickpea seed and straw yield have been reported by Swetha and Hussain (2017) and Sarkar et al. (2016). Improvement in root-zone soil moisture through irrigation favoured plant height, dry-matter accumulation and yield-attributing characters, thereby enhancing biological yield, while VAM enhanced the uptake of essential nutrients such as phosphorus and nitrogen that support plant growth and biomass production; this is consistent with the comparatively smaller reduction in straw yield recorded in JAKI-9218 relative to the other genotypes.

Table 1

Seed yield and straw yield (kg ha⁻¹) of chickpea as influenced by water regime and variety, Rabi 2022-23, 2023-24 and pooled analysis.

Treatment	Seed yield (kg ha ⁻¹)			Straw yield (kg ha ⁻¹)		
	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
Water regime (A)						
Irrigated	1632.71	1671.71	1652.21	2091.43	2103.00	2097.21
Moisture stress	1407.29	1415.29	1411.29	1820.71	1832.86	1826.79
SEm±	36.33	36.96	36.65	46.67	46.95	46.81
CD (5%)	105.59	107.44	106.51	135.66	136.47	136.06
Variety (B)						
PDKV Kanak	1832.00	1842.50	1837.25	2317.00	2329.00	2323.00
PDKV Kanchan	1764.50	1769.50	1767.00	2248.50	2262.00	2255.25
PKV Kabuli-2	1187.00	1216.00	1201.50	1559.50	1577.50	1568.50
PKV Kabuli-4	1109.00	1148.00	1128.50	1448.50	1444.00	1446.25
JAKI-9218	1711.00	1740.50	1725.75	2151.50	2159.00	2155.25
Chandrapur Chanoli	1405.00	1421.00	1413.00	1825.00	1833.50	1829.25
Gulak-1	1631.50	1667.00	1649.25	2142.50	2170.50	2156.50
SEm±	67.96	69.15	68.56	87.32	87.84	87.58
CD (5%)	197.54	201.00	199.26	253.79	255.31	254.55
Interaction (A × B)						
SEm±	96.12	97.80	96.95	123.49	124.22	123.85
CD (5%)	279.36	284.25	281.80	358.91	361.06	359.98

3.2 Chlorophyll a, chlorophyll b and total chlorophyll content

Total chlorophyll content was recorded at 30, 60 and 90 days after sowing (DAS) (Table 2; Fig. 2a-c). Chlorophyll content, irrespective of water regime and variety, increased from 30 DAS to a peak at 60 DAS and then declined by 90 DAS. The effect of water regime on total chlorophyll content was non-significant at 30 and 60 DAS in both seasons and in the pooled analysis, but became significant at 90 DAS: irrigated plants recorded significantly higher total chlorophyll content (1.32 mg g⁻¹ FW, pooled) than moisture-stressed plants (1.10 mg g⁻¹ FW). Varietal differences followed the same pattern, becoming significant only at 90 DAS, when JAKI-9218 recorded the highest total chlorophyll content (1.42 mg g⁻¹ FW),

statistically at par with PDKV Kanak (1.32 mg g⁻¹ FW) and PDKV Kanchan (1.32 mg g⁻¹ FW), while PKV Kabuli-2 recorded the lowest (1.01 mg g⁻¹ FW).

The water regime × variety interaction for total chlorophyll content was likewise significant only at 90 DAS (Table 3; Fig. 2d). Under irrigated conditions, JAKI-9218 recorded the highest total chlorophyll content (1.52 mg g⁻¹ FW, pooled) and PKV Kabuli-2 the lowest (1.12 mg g⁻¹ FW); under moisture stress, JAKI-9218 again recorded the highest value (1.31 mg g⁻¹ FW), statistically at par with PDKV Kanchan (1.22 mg g⁻¹ FW) and PDKV Kanak (1.21 mg g⁻¹ FW), while PKV Kabuli-2 recorded the lowest (0.91 mg g⁻¹ FW). The smallest percentage reduction in total chlorophyll content under moisture stress was recorded in JAKI-9218 (13.8%), followed by PDKV Kanchan (14.7%) and PDKV Kanak (16.0%), while Gulak-1 showed one of the largest reductions (19.5%), together with PKV Kabuli-4 (20.2%).

The decline in chlorophyll content beyond 60 DAS may partly reflect a reduced light-harvesting capacity that limits the generation of reactive oxygen species through degradation of the light-absorbing pigments, and may also be associated with premature leaf senescence induced by heat and moisture stress, which significantly reduces the photosynthetic rate at later growth stages. VAM enhances the uptake of nutrients and water — especially phosphorus — thereby helping to sustain photosynthetic capacity and chlorophyll content under stress. Similar findings were reported by Karim et al. (2022) and Keerthi Sree et al. (2023).

Relative water content (%) was recorded at 30, 60 and 90 DAS (Table 2; Fig. 3a). The effect of water regime on RWC was non-significant at 30 DAS in both seasons and in the pooled analysis, but significant differences emerged at 60 and 90 DAS. At 90 DAS, irrigated plants recorded a significantly higher RWC (67.62%, pooled) than moisture-stressed plants (53.21%). Varietal differences in RWC were likewise non-significant at 30 DAS but significant at 60 and 90 DAS: at 90 DAS, JAKI-9218 recorded the highest RWC (68.03%), statistically at par with PDKV Kanak, PDKV Kanchan and Chandrapur Chanoli, while Gulak-1 recorded the lowest (54.87%).

The water regime × variety interaction for RWC was significant at 90 DAS (Table 3; Fig. 3b). Under irrigated conditions, JAKI-9218 recorded the highest RWC (73.67%, pooled) and Gulak-1 the lowest (62.97%); under moisture stress, JAKI-9218 again recorded the highest RWC (62.39%), statistically at par with PDKV Kanak, PDKV Kanchan and Chandrapur Chanoli, while Gulak-1 recorded the lowest (46.76%). The smallest percentage reduction in RWC under moisture stress was recorded in JAKI-9218 (15.3%), followed by PDKV Kanak (19.9%) and PDKV Kanchan (20.2%), while Gulak-1 recorded the largest reduction (25.7%).

The progressive decline in RWC from 30 DAS onwards under moisture stress reflects the increasing water scarcity experienced by the crop as the season advances, which limits transpiration and other physiological processes and reduces leaf water content. VAM can help alleviate this decline by enhancing water uptake, increasing hydraulic conductivity and promoting root development, thereby improving water absorption and transport; under stress, VAM may act as a kind of "water reservoir" for the plant, while under irrigated conditions it can help improve water-use efficiency. The consistently

smaller reduction in RWC recorded in JAKI-9218 indicates that this genotype is more stress-tolerant, followed by PDKV Kanak and PDKV Kanchan, whereas Gulak-1 appears to be the most susceptible variety. These findings agree with those of Islam et al. (2021) and Meddich et al. (2021).

3.4 Proline content

Proline content was recorded at 50% flowering and at the pod-filling stage (Table 2; Fig. 3c and 3d). Water-regime conditions had a significant effect on proline content at both growth stages in both seasons and in the pooled analysis. At 50% flowering, moisture-stressed plants accumulated significantly more proline ($10.71 \mu\text{mol g}^{-1} \text{FW}$, pooled) than irrigated plants ($5.50 \mu\text{mol g}^{-1} \text{FW}$); at the pod-filling stage the difference was even larger, with moisture-stressed plants recording $16.36 \mu\text{mol g}^{-1} \text{FW}$ against $6.12 \mu\text{mol g}^{-1} \text{FW}$ under irrigation. Varietal differences in proline content were significant at both stages: at 50% flowering, JAKI-9218 recorded the highest proline content ($10.74 \mu\text{mol g}^{-1} \text{FW}$), statistically at par with PDKV Kanak ($9.49 \mu\text{mol g}^{-1} \text{FW}$) and PDKV Kanchan ($9.03 \mu\text{mol g}^{-1} \text{FW}$), while Gulak-1 recorded the lowest ($6.23 \mu\text{mol g}^{-1} \text{FW}$); a similar ranking held at pod filling, with JAKI-9218 again highest ($13.57 \mu\text{mol g}^{-1} \text{FW}$) and Gulak-1 lowest ($9.01 \mu\text{mol g}^{-1} \text{FW}$).

The water regime $\times$ variety interaction for proline content was significant at both stages (Table 3). Under moisture stress at the pod-filling stage, JAKI-9218 accumulated significantly more proline ($20.18 \mu\text{mol g}^{-1} \text{FW}$, pooled) than any other genotype, followed by PDKV Kanak ($19.20 \mu\text{mol g}^{-1} \text{FW}$) and PDKV Kanchan ($18.36 \mu\text{mol g}^{-1} \text{FW}$), while Gulak-1 recorded the lowest accumulation ($12.61 \mu\text{mol g}^{-1} \text{FW}$). Expressed relative to the irrigated control, JAKI-9218 showed the largest proportional increase in proline content at pod filling (65.5% of the stressed-plant value, i.e. the stressed mean exceeded the irrigated mean by roughly two-thirds of itself), followed by PDKV Kanak and PDKV Kanchan, while Gulak-1 showed the smallest proportional increase (57.0%), indicating that JAKI-9218 is the more drought-tolerant genotype and Gulak-1 the more susceptible one – consistent with the pattern already seen for yield, chlorophyll content and RWC.

Under drought stress, water deficit induces the accumulation of proline, which acts as an osmoregulatory solute that helps maintain cell turgor and protects cell membranes from damage; this accumulation is typically more pronounced in drought-tolerant cultivars than in susceptible ones, which is consistent with the strong proline build-up observed here in JAKI-9218. These results agree with the findings of Yooyongwech et al. (2023), who similarly reported a role for proline in imparting drought tolerance in legumes under AMF inoculation.

Table 2

Physiological and biochemical traits of chickpea as influenced by water regime and variety, pooled analysis of Rabi 2022-23 and 2023-24.

Treatment	Total chlorophyll 90 DAS (mg g⁻¹ FW)	RWC 90 DAS (%)	Proline 50% flowering (μmol g⁻¹ FW)	Proline pod filling (μmol g⁻¹ FW)	SOD activity pod filling (U mg⁻¹ protein)
Water regime (A)					
Irrigated	1.32	67.62	5.50	6.12	55.00
Moisture stress	1.10	53.21	10.71	16.36	84.32
CD (5%)	0.09	4.36	0.64	0.93	5.29
Variety (B)					
PDKV Kanak	1.32	63.96	9.49	13.04	64.23
PDKV Kanchan	1.32	62.93	9.03	12.55	67.52
PKV Kabuli-2	1.01	56.16	6.84	10.03	76.71
PKV Kabuli-4	1.07	56.36	6.50	9.21	79.79
JAKI-9218	1.42	68.03	10.74	13.57	62.81
Chandrapur Chanoli	1.18	60.62	7.91	11.28	67.30
Gulak-1	1.15	54.87	6.23	9.01	69.27
CD (5%)	0.16	8.16	1.20	1.74	9.89

3.5 Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was recorded at the pod-filling stage (Table 2; Fig. 4a). Water-regime conditions had a significant effect on SOD activity in both seasons and in the pooled analysis: moisture-stressed plants recorded significantly higher SOD activity (84.32 U mg⁻¹ protein, pooled) than irrigated plants (55.00 U mg⁻¹ protein). Varietal effects were also significant: PKV Kabuli-4 recorded the numerically highest SOD activity (79.79 U mg⁻¹ protein), followed by PKV Kabuli-2 and PDKV Kanchan, while PDKV Kanak recorded the numerically lowest (64.23 U mg⁻¹ protein). However, when expressed as the percentage increase in SOD activity under moisture stress relative to irrigated conditions, JAKI-9218 showed by far the largest induction (86.7%), followed by PDKV Kanak (70.0%) and PDKV Kanchan (64.9%), while Gulak-1 showed the smallest induction (31.9%).

The water regime × variety interaction for SOD activity was significant (Table 3). Under irrigated conditions, PKV Kabuli-4 recorded the highest SOD activity ($67.64 \text{ U mg}^{-1} \text{ protein}$, pooled) and JAKI-9218 the lowest ($43.82 \text{ U mg}^{-1} \text{ protein}$); under moisture stress, PKV Kabuli-4 again recorded the highest SOD activity ($91.94 \text{ U mg}^{-1} \text{ protein}$), followed by PKV Kabuli-2 ($89.02 \text{ U mg}^{-1} \text{ protein}$) and PDKV Kanchan ($84.05 \text{ U mg}^{-1} \text{ protein}$), while Gulak-1 recorded the lowest ($78.81 \text{ U mg}^{-1} \text{ protein}$).

VAM fungi can enhance SOD activity in chickpea, particularly under water-stress conditions, by strengthening antioxidant defence mechanisms, which confers better resilience to the oxidative stress induced by water scarcity. The markedly larger relative induction of SOD activity in JAKI-9218 under moisture stress, despite its comparatively moderate absolute SOD values, indicates a more responsive antioxidative system in this genotype that complements its superior proline accumulation, chlorophyll retention and water-status maintenance, together explaining its overall drought tolerance. Similar results were obtained by Sharma (2016) and Azizoglu et al. (2025).

3.6 Comparative drought tolerance among chickpea genotypes

Taken together, the six traits examined present a consistent picture of genotypic differences in drought response (Fig. 4b). Across seed yield, straw yield, total chlorophyll content and relative water content, JAKI-9218 consistently showed the smallest percentage decline under moisture stress relative to irrigated conditions (10.3%, 9.8%, 13.8% and 15.3%, respectively), while also showing the largest relative induction of the protective, stress-responsive traits — proline accumulation and SOD activity. PDKV Kanak and PDKV Kanchan followed a broadly similar, though somewhat less pronounced, pattern, while PKV Kabuli-2, PKV Kabuli-4 and Gulak-1 combined comparatively larger reductions in yield and water-related traits with weaker antioxidative and osmotic adjustment, marking them as the more drought-susceptible genotypes among those tested; Gulak-1 in particular recorded the largest reduction in seed yield, straw yield and relative water content of all seven genotypes. This convergence of a favourable yield response with favourable physiological and biochemical indicators in JAKI-9218 — and, to a lesser degree, in PDKV Kanak and PDKV Kanchan — under VAM inoculation identifies these genotypes as promising material both for direct cultivation in drought-prone tracts and as parental lines in breeding programmes aimed at improving terminal-drought tolerance in chickpea.

Table 3

Interaction effect of water regime × variety on yield and physio-biochemical traits of chickpea, pooled analysis of Rabi 2022-23 and 2023-24.

Water regime	PDKV Kanak	PDKV Kanchan	PKV Kabuli-2	PKV Kabuli-4	JAKI-9218	Chandrapur Chanoli	Gulak-1
Seed yield (kg ha⁻¹)							
Irrigated	1961.50	1894.00	1313.00	1226.00	1819.50	1517.50	1834.00
Moisture stress	1713.00	1640.00	1090.00	1031.00	1632.00	1308.50	1464.50
Straw yield (kg ha⁻¹)							
Irrigated	2451.00	2383.00	1699.50	1569.00	2266.00	1942.00	2370.00
Moisture stress	2195.00	2127.50	1437.50	1323.50	2044.50	1716.50	1943.00
Total chlorophyll content at 90 DAS (mg g⁻¹ FW)							
Irrigated	1.44	1.43	1.12	1.19	1.52	1.28	1.28
Moisture stress	1.21	1.22	0.91	0.95	1.31	1.08	1.03
Relative water content at 90 DAS (%)							
Irrigated	71.04	70.02	63.84	63.94	73.67	67.89	62.97
Moisture stress	56.89	55.85	48.49	48.77	62.39	53.35	46.76
Proline content at 50% flowering (μmol g⁻¹ FW)							
Irrigated	6.62	6.17	4.68	4.49	6.92	5.31	4.32
Moisture stress	12.37	11.90	9.00	8.51	14.56	10.51	8.15
Proline content at pod-filling stage (μmol g⁻¹ FW)							
Irrigated	6.89	6.73	5.58	5.14	6.96	6.13	5.42
Moisture stress	19.20	18.36	14.48	13.28	20.18	16.44	12.61
SOD activity at pod-filling stage (U mg⁻¹ protein)							
Irrigated	47.58	50.98	64.40	67.64	43.82	50.84	59.73
Moisture stress	80.89	84.05	89.02	91.94	81.80	83.76	78.81

CD (5%) for the water regime × variety interaction effect (pooled analysis): seed yield = 281.80 kg ha⁻¹; straw yield = 359.98 kg ha⁻¹; total chlorophyll content (90 DAS) = 0.23 mg g⁻¹ FW; relative water content (90 DAS) = 11.54; proline content at 50% flowering = 1.70 μmol g⁻¹ FW; proline content at pod filling = 2.46 μmol g⁻¹ FW; SOD activity at pod filling = 13.99 U mg⁻¹ protein.

4. Conclusion

Terminal moisture stress significantly reduced seed yield, straw yield, total chlorophyll content and relative water content of chickpea, while significantly increasing proline accumulation and superoxide dismutase activity, across two years of field evaluation of seven chickpea genotypes grown with VAM inoculation. Among the genotypes tested, JAKI-9218 consistently combined the smallest relative decline in yield and water-related traits with the strongest osmotic and antioxidative adjustment under stress, identifying it as the most drought-tolerant genotype, followed by PDKV Kanak and PDKV Kanchan, while Gulak-1 was the most drought-susceptible. These results suggest that VAM inoculation, together with the deployment of inherently tolerant genotypes such as JAKI-9218, offers a practical, input-efficient strategy to sustain chickpea productivity in drought-prone, rainfed tracts, and that JAKI-9218, PDKV Kanak and PDKV Kanchan merit further evaluation as donor parents in breeding programmes targeting improved terminal-drought tolerance in chickpea.

Declarations

Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability Statement:

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Asati R, Tripathi MK, Yadav RK, Tripathi N, Sikarwar RS, Tiwari PN (2024) Investigation of drought stress on chickpea (*Cicer arietinum* L.) genotypes employing various physiological enzymatic and non-enzymatic biochemical parameters. *Plants* 13:2746. <https://doi.org/10.3390/plants13192746>
2. Azizoglu U, Argenteel-Martínez L, Peñuelas-Rubio O, Meriño-Hernández Y, Rodríguez-Yon Y, Amico-Rodríguez D, J.M. and, Shin JH (2025) Arbuscular mycorrhizal fungus *Rhizophagus irregularis* enhances antioxidant system mechanisms in chickpea plants under saline conditions. *Plant Growth Regul* 1–12. <https://doi.org/10.1007/s10725-025-01315-5>
3. Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian J Biol Sci* 15:413–428. <https://doi.org/10.1071/BI9620413>
4. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207. <https://doi.org/10.1007/BF00018060>
5. Berruti A, Borriello R, Orgiazzi A, Barbera AC, Lumini E, Bianciotto V (2014) Arbuscular mycorrhizal fungi and their value for ecosystem management. In: Grillo, O. (ed.) *Biodiversity: The Dynamic Balance of the Planet*. InTech, pp. 159–191. <https://doi.org/10.5772/58231>
6. Boutasknit A, Baslam M, Ait-El-Mokhtar M, Anli M, Ben-Laouane R, Douira A, Modafar E, Mitsui C, Wahbi T, S. and, Meddich A (2020) Arbuscular mycorrhizal fungi mediate drought tolerance and recovery in two contrasting carob (*Ceratonia siliqua* L.) ecotypes by regulating stomatal, water relations, and (in)organic adjustments. *Plants* 9:80. <https://doi.org/10.3390/plants9010080>
7. Chandrasekaran M (2024) The role of arbuscular mycorrhizal fungi in refining plant photosynthesis and water status under drought stress: a meta-analysis. *Plant Soil Environ* 70:502–508. <https://doi.org/10.17221/27/2024-PSE>
8. DES (2023) Department of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India. No DOI. government statistical report.)
9. FAOSTAT, Agriculture Organization of the United Nations (2023) Food and. Available at: <https://www.fao.org/faostat/en/home> (Accessed 22 December 2023). (No DOI; online statistical database.)
10. Ferioun M, Zouitane I, Bouhraoua S, Elouattassi Y, Belahcen D, Errabbani A, Louahlia S, Sayyed R, Ghachtouli E, N (2025) Applying microbial biostimulants and drought-tolerant genotypes to enhance barley growth and yield under drought stress. *Front Plant Sci* 15:1494987. <https://doi.org/10.3389/fpls.2024.1494987>
11. Gaur PM, Jukanti AK, Samineni S, Chaturvedi SK, Basu PS, Babbar A, Jayalakshmi V, Nayyar H, Devasirvatham V, Mallikarjuna N, Krishnamurthy L, Gowda L, C.L (2013) Climate change and heat stress tolerance in chickpea. In: Tuteja N, Gill SS (eds) *Climate Change and Plant Abiotic Stress Tolerance*. Wiley-VCH. <https://doi.org/10.1002/9783527675265.ch31>
12. Havargi SS (2007) [Full bibliographic details were not provided in the source manuscript, and could not be independently verified from the citation as given; please supply the correct journal/thesis

details and DOI, if any, before submission.]

13. Islam MR, Kamal MM, Alam MA, Hossain J, Soufan W, Skalicky M, Brestic M, Abderrahman M, Saneoka H, Islam MS (2021) Physiochemical changes of mung bean (*Vigna radiata* (L.) R. Wilczek) in response to varying irrigation regimes. *Horticulturae* 7:565. <https://doi.org/10.3390/horticulturae7120565>
14. Istanbuli T, Alsamman AM, Al-Shamaa K, Abu Assar A, Adlan M, Kumar T, Tawkaz S, Hamwieh A (2025) Selection of high nitrogen fixation chickpea genotypes under drought stress conditions using multi-environment analysis. *Front Plant Sci* 16:1490080. <https://doi.org/10.3389/fpls.2025.1490080>
15. Itam M et al (2020) [Full bibliographic details were not provided in the source manuscript, and could not be independently verified from the citation as given; please supply the correct journal details and DOI, if any, before submission.]
16. Jabborova D, Annapurna K, Azimov A, Tyagi S, Pengani KR, Sharma P, Vikram KV, Poczai P, Nasif O, Ansari MJ, Sayyed RZ (2022) Co-inoculation of biochar and arbuscular mycorrhizae for growth promotion and nutrient fortification in soybean under drought conditions. *Front Plant Sci* 13:947547. <https://doi.org/10.3389/fpls.2022.947547>
17. Jerbi M, Labidi S, Laruelle F, Tisserant B, Dalpé Y, Lounès-Hadj Sahraoui A, Ben Jeddi F (2022) Contribution of native and exotic arbuscular mycorrhizal fungi in improving the physiological and biochemical response of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) to drought. *J Soil Sci Plant Nutr* 22:2187–2204. <https://doi.org/10.1007/s42729-022-00802-2>
18. Jha UC, Chaturvedi SK, Bohra A, Basu PS, Khan MS, Barh D (2014) Abiotic stresses, constraints and improvement strategies in chickpea. *Plant Breeding* 133:163–178. <https://doi.org/10.1111/pbr.12150>
19. Kaloki P, Luo Q, Trethowan R, Tan DK (2019) Can the development of drought-tolerant ideotype sustain Australian chickpea yield? *Int J Biometeorol* 63:393–403. <https://doi.org/10.1007/s00484-019-01672-7>
20. Karim AMA, Sarker UK, Hasan AK, Islam N, Uddin MR (2022) Physiological and biochemical responses of chickpea genotypes to different moisture stress in Bangladesh. *Turkish J Field Crops* 27:1–9 (No DOI assigned by the journal.)
21. Karkee A, Barmukh R, Akinlade O, Kotte B, Norton SL, Hobson K, Li C, Kamphuis L, Sadras VO, Varshney RK (2026) Advances, challenges, and opportunities to improve drought tolerance in chickpea. *Plant Stress* 21:101406. <https://doi.org/10.1016/j.stress.2026.101406>
22. Keerthi Sree Y, Lakra N, Manorama K, Ahlawat Y, Zaid A, Elansary HO, Mahmoud EA (2023) Drought-induced morpho-physiological, biochemical, metabolite responses and protein profiling of chickpea (*Cicer arietinum* L.). *Agronomy* 13:1814. <https://doi.org/10.3390/agronomy13071814>
23. Kumar P, Boora KS, Kumar N, Ritu B (2018) Traits of significance for screening of chickpea (*Cicer arietinum* L.) genotypes under terminal drought stress. *J Agrometeorology* 20:40–45. <https://doi.org/10.54386/jam.v20i1.501>

24. Kushwah A, Bhatia D, Singh G, Singh I, Bindra S, Vij S, Singh S (2021) Phenotypic evaluation of genetic variability and selection of yield contributing traits in chickpea recombinant inbred line population under high temperature stress. *Physiol Mol Biology Plants* 27:747–767.
<https://doi.org/10.1007/s12298-021-00977-5>
25. Maqbool MA, Aslam M, Ali H (2017) Breeding for improved drought tolerance in chickpea (*Cicer arietinum* L). *Plant Breeding* 136:300–318. <https://doi.org/10.1111/pbr.12477>
26. Meddich A, Ouhammadou R, Anli M, Boutasknit A (2021) Role of phosphorus and arbuscular mycorrhizal fungi in the growth performance and tolerance of barley to water stress. *Plant Cell Biotechnol Mol Biology* 22:45–67 (No DOI located for this article.)
27. Nader AA, Hauka FIA, Afify AH, El-Sawah AM (2024) Drought-tolerant bacteria and arbuscular mycorrhizal fungi mitigate the detrimental effects of drought stress induced by withholding irrigation at critical growth stages of soybean (*Glycine max* L). *Microorganisms* 12:1123.
<https://doi.org/10.3390/microorganisms12061123>
28. Niveditha MP, Patil SB, Kalaghatagi SB, Ashvathama VH (2022) Growth and yield of chickpea genotypes under changing weather scenario in the Northern Dry Zone of Karnataka. *J Farm Sci* 35:192–198 (No DOI located for this article.)
29. Rani A et al (2020) [Full bibliographic details were not provided in the source manuscript, and could not be independently verified from the citation as given; please supply the correct journal details and DOI, if any, before submission.]
30. Rizvi AH, Dwivedi VK, Sairam RK, Yadav SS, Bharadwaj C, Sarker A, Alam A (2014) Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes. *Int J Sci Res Agricultural Sci* 1:23–31 (No DOI located for this article.)
31. Ryan JG (1997) A global perspective on pigeonpea and chickpea sustainable production systems: present status and future potential. In: Asthana, A.N. and Ali, M. (eds.) *Recent Advances in Pulses Research*. Indian Society of Pulses Research and Development, Kanpur, pp. 1–31. (No DOI; pre-DOI-era conference proceedings volume.)
32. Sachdeva S, Bharadwaj C, Patil BS, Pal M, Roorkiwal M, Varshney RK (2022) Agronomic performance of chickpea affected by drought stress at different growth stages. *Agronomy* 12:995.
<https://doi.org/10.3390/agronomy12050995>
33. Sadasivam S, Manickam A (1996) *Biochemical Methods for Agricultural Sciences*. New Age International, New Delhi. (No DOI; book.)
34. Sarkar S, Sarkar A, Zaman A (2016) Yield, water use and economics of chickpea (*Cicer arietinum*) as influenced by different levels of irrigation and mulches. *Ind J Agron* 61:479–483 (No DOI located for this article.)
35. Sharma P et al (2012) [Full bibliographic details were not provided in the source manuscript, and could not be independently verified from the citation as given; please supply the correct journal details and DOI, if any, before submission.]

36. Sharma V (2016) Physiological studies on moisture stress tolerance in chickpea (*Cicer arietinum* L.) genotypes. Doctoral dissertation, Punjab Agricultural University, Ludhiana. (No DOI; doctoral dissertation.)
37. Singh V, Gupta K, Singh S, Jain M, Garg R (2023) Unravelling the molecular mechanism underlying drought stress response in chickpea via integrated multi-omics analysis. *Front Plant Sci* 14:1156606. <https://doi.org/10.3389/fpls.2023.1156606>
38. Swetha D, Hussain SA (2017) Effect of moisture regimes on yield attributes, yield, nutrient uptake and quality of chickpea cultivars (*Cicer arietinum* L). *Int J Curr Microbiol Appl Sci* 6:3636–3645 (No DOI located for this article.)
39. Tiwari PN, Tiwari S, Sapre S, Babbar A, Tripathi N, Tiwari S, Tripathi MK (2023) Screening and selection of drought-tolerant high-yielding chickpea genotypes based on physio-biochemical selection indices and yield trials. *Life* 13:1405. <https://doi.org/10.3390/life13061405>
40. Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Tar'an B et al (2013) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat Biotechnol* 31:240–246. <https://doi.org/10.1038/nbt.2491>
41. Vishnu B, Jayalakshmi V, Rani Sudha M (2018) Genetic diversity studies among chickpea (*Cicer arietinum* L.) genotypes under rainfed and irrigated conditions for yield-attributing traits and traits related to mechanical harvesting. *Legume Res* 43:190–194. <https://doi.org/10.18805/LR-3959>
42. Wallace TC, Murray R, Zelman KM (2016) The nutritional value and health benefits of chickpeas and hummus. *Nutrients* 8:766. <https://doi.org/10.3390/nu8120766>
43. Yimer T, Abera G, Beyene S, Bono B, Rasche F (2024) Combined inoculation of arbuscular mycorrhiza fungi with *Mesorhizobium* improves nutrient uptake, growth performance, and moisture stress tolerance of chickpea (*Cicer arietinum* L). *Agrosystems Geosci Environ* 7:e20562. <https://doi.org/10.1002/agg2.20562>
44. Yooyongwech S, Tisarum R, Samphumphuang T, Phisalaphong M, Cha-um S (2023) Integrated strength of osmotic potential and phosphorus to achieve grain yield of rice under water deficit by arbuscular mycorrhiza fungi. *Sci Rep* 13:5999. <https://doi.org/10.1038/s41598-023-33304-x>
45. Zargar SM, Khan T, Mir RA, Zaffar A, Urwat U, Rashid M, Sofi PA, Afroz B, Deveshwar P, Rakwal R, Ali S (2026) Integrating multi-omics approaches to shape legume root system architecture under drought stress: a comprehensive review. *Front Plant Sci* 17:1783318. <https://doi.org/10.3389/fpls.2026.1783318>

Figures

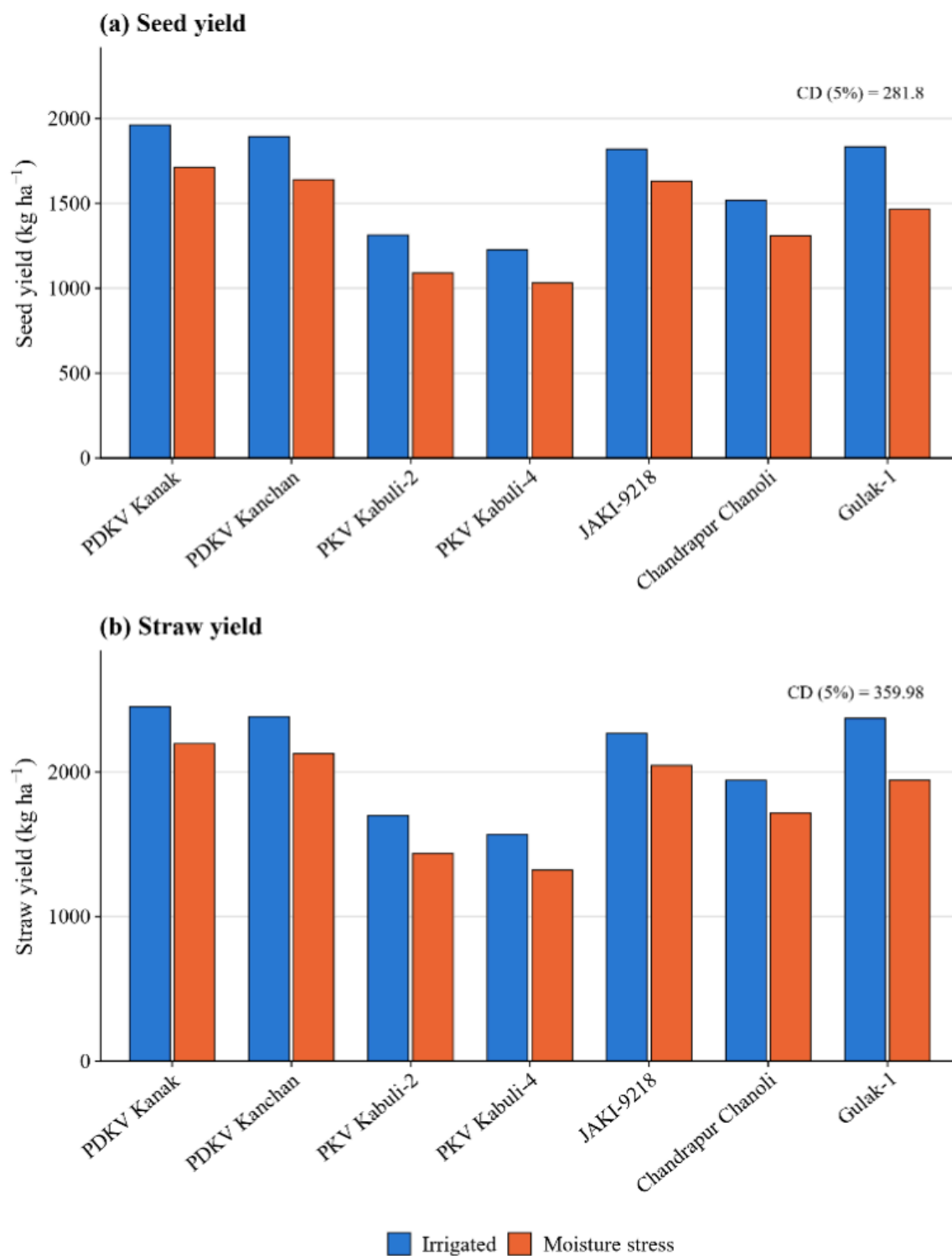


Figure 1

Seed yield (a) and straw yield (b) of chickpea as influenced by the interaction of water regime and variety (pooled analysis, Rabi 2022-23 and 2023-24). Error terms: CD (5%) = 281.80 kg ha⁻¹ (seed yield) and 359.98 kg ha⁻¹ (straw yield).

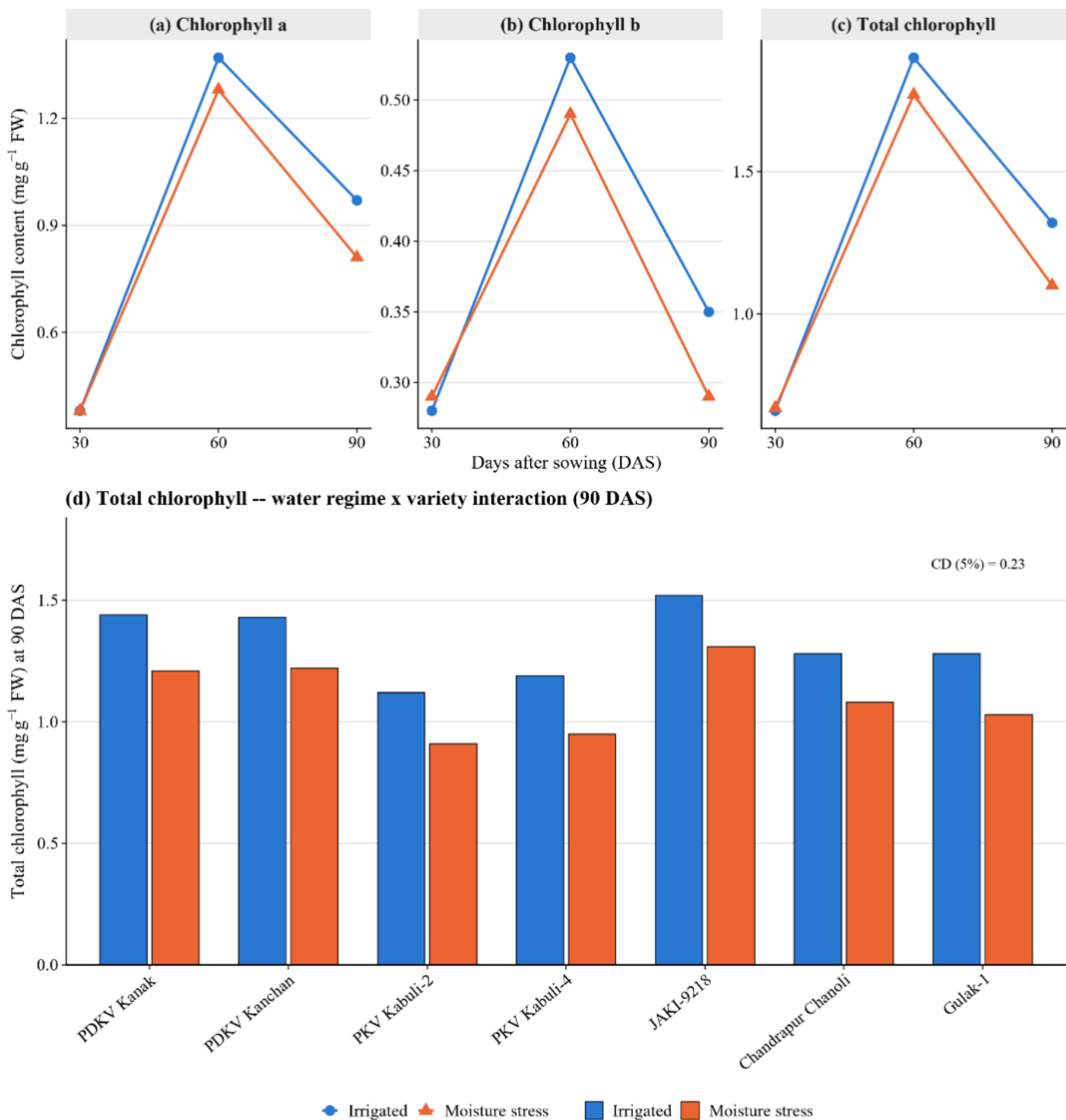


Figure 2

Chlorophyll a (a), chlorophyll b (b) and total chlorophyll content (c) (mg g⁻¹ FW) of chickpea at 30, 60 and 90 DAS under irrigated and moisture-stress conditions (pooled analysis), and the interaction effect of water regime and variety on total chlorophyll content at 90 DAS (d; CD (5%) = 0.23 mg g⁻¹ FW). 3.3 Relative water content (RWC)

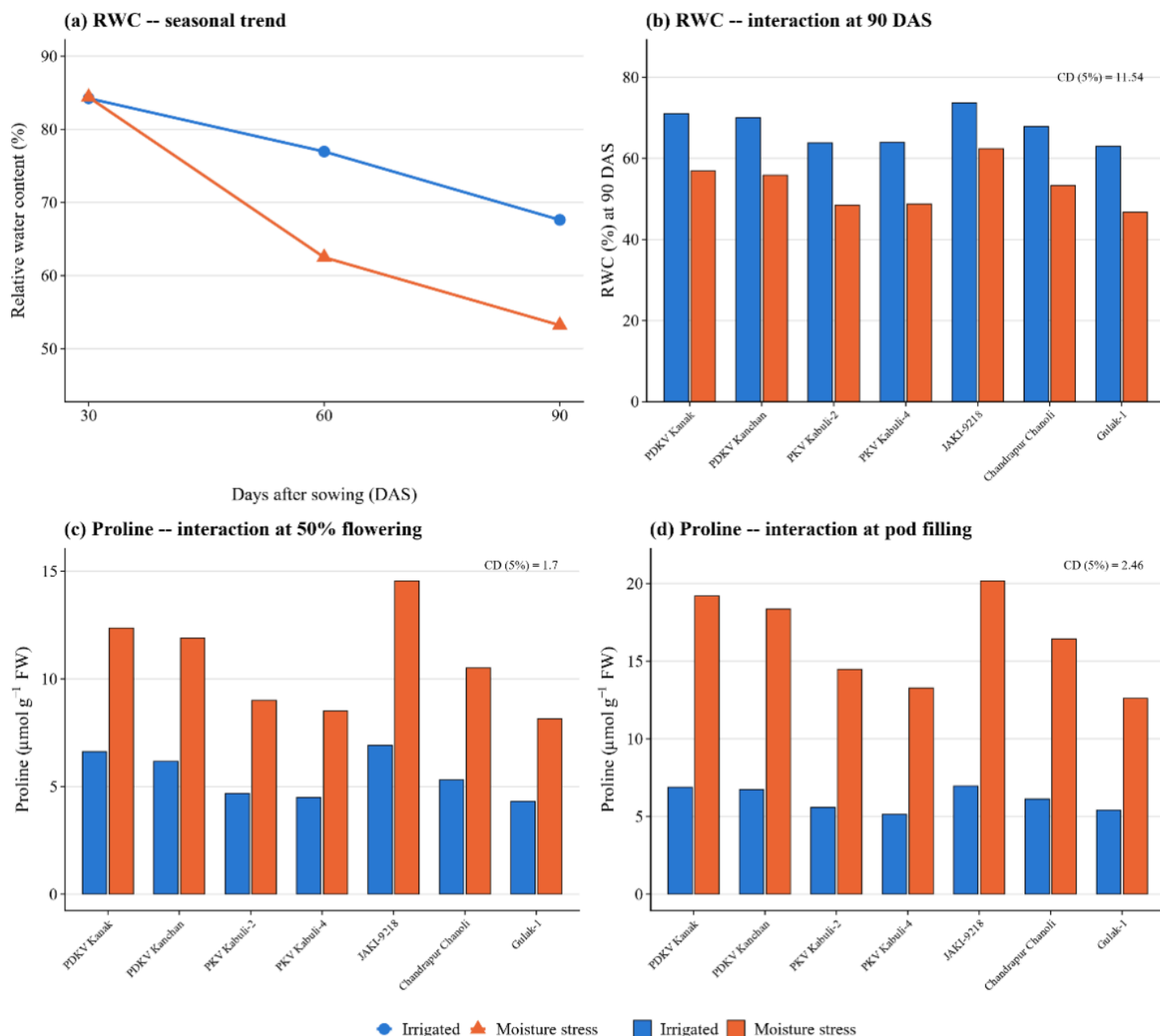


Figure 3

Relative water content (%) of chickpea at 30, 60 and 90 DAS under irrigated and moisture-stress conditions (a), the interaction effect of water regime and variety on relative water content at 90 DAS (b; CD (5%) = 11.54), and the interaction effect of water regime and variety on proline content ($\mu\text{mol g}^{-1} \text{FW}$) at 50% flowering (c; CD (5%) = 1.70) and at the pod-filling stage (d; CD (5%) = 2.46) (pooled analysis).

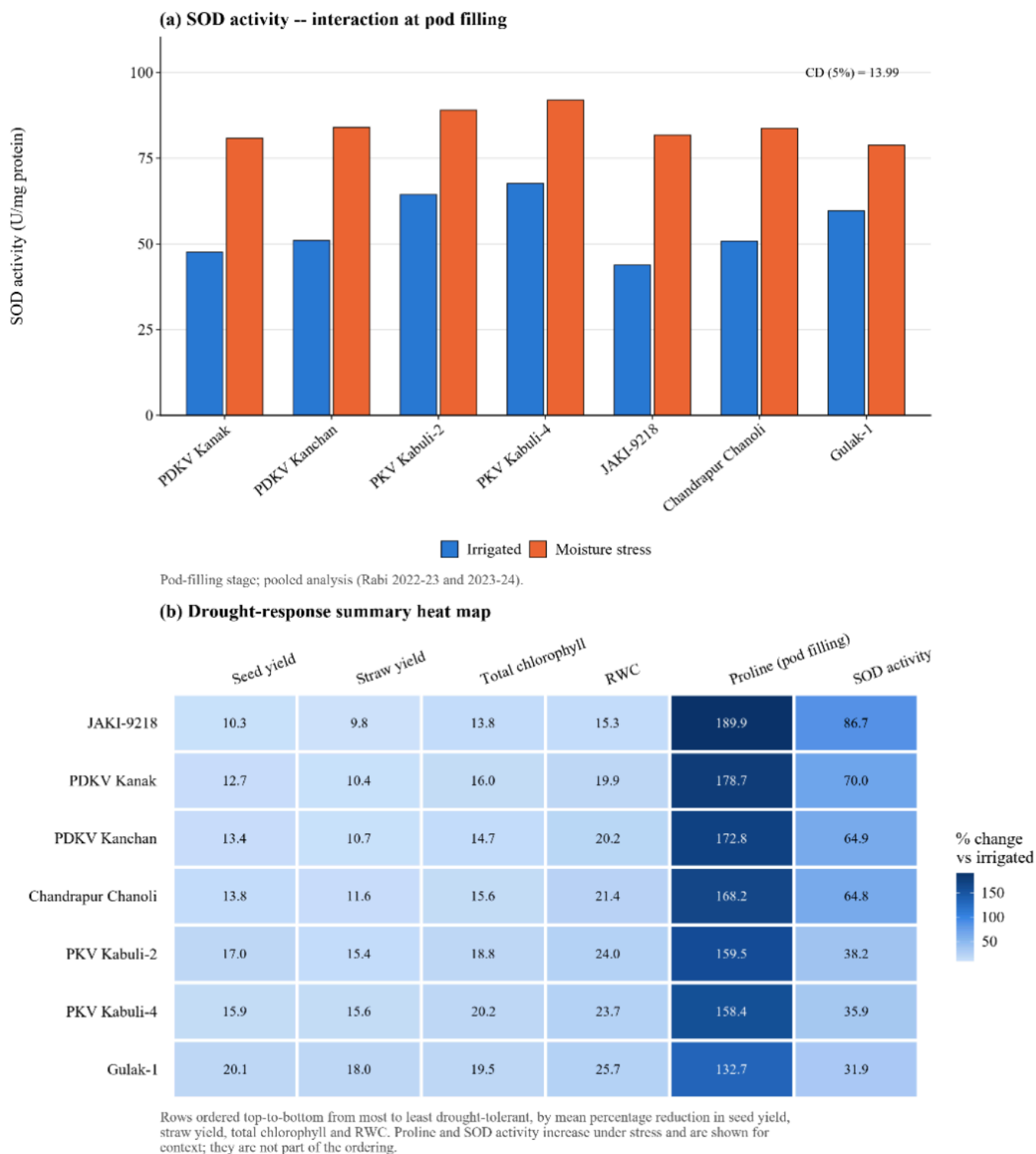


Figure 4

Interaction effect of water regime and variety on superoxide dismutase (SOD) activity (U mg^{-1} protein) at the pod-filling stage (a; CD (5%) = 13.99), and a drought-response summary heat map (b) showing percentage change in seed yield, straw yield, total chlorophyll content and relative water content (reduction), and in proline content and SOD activity (increase), under moisture stress relative to

irrigated conditions, by variety (pooled analysis); varieties are ordered from most to least drought-tolerant by mean percentage reduction in the four yield/physiological traits.