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Physiological determinants of drought tolerance in groundnut (*Arachis hypogaea* L.): insights from multi-generational responses

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Abstract
Drought stress induces considerable variation in core physiological traits that regulate photosynthetic efficiency, water-use dynamics and adaptive responses in groundnut. Understanding the inheritance of these traits across generations is essential for developing drought-resilient cultivars. The present study evaluated five key physiological determinants *viz*., SPAD chlorophyll meter reading, water-use efficiency (WUE), specific leaf area (SLA), number of stomata and stomatal conductance, across six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) under irrigated and drought-stress conditions. Generation mean analysis was employed to assess the magnitude of genetic effects governing the inheritance of these traits.

Analysis of variance revealed significant differences among all six generations under both environments, highlighting the availability of substantial physiological variability. F_1 means were mostly intermediate or superior to both parents, indicating partial to complete dominance for several traits related to chlorophyll retention, stomatal behaviour and leaf structural characteristics. Under irrigated conditions, the high-yielding parent performed better for SLA and stomatal conductance, while the drought-tolerant parent exhibited superior SPAD values and WUE under stress. BC_2 (F_1 × ICG 4670) recorded the highest SPAD, WUE and optimal stomatal adjustment under drought, whereas BC_1 (F_1 × TAG-24) demonstrated better performance under irrigation.

The simple additive dominance model was insufficient; therefore, a six-parameter model was used. Significant A, B, C and D scaling tests and joint scaling test results indicated the presence of epistatic interactions. Under irrigated conditions, dominance (h) and dominance × dominance (l) interactions were prominent for SLA, stomatal conductance and number of stomata, suggesting the prevalence of non-additive gene action. Under drought stress, dominance and dominance × dominance effects again dominated for SPAD and WUE, traits central to maintaining photosynthetic stability and water-use efficiency under limited moisture. These outcomes suggest that hybridization followed by delayed selection would be more effective for improving non-additive traits.

Additive (d) and additive × additive (i) gene effects were significant for WUE, SPAD and number of stomata, indicating that early-generation selection could be feasible for traits with more fixable inheritance. Duplicate epistasis was more frequent than complementary epistasis under both environments, implying that mild selection in early generations and stronger selection pressure in advanced generations would enhance breeding progress.

Overall, this study emphasizes the importance of integrating key physiological traits, particularly SPAD, WUE and stomatal behaviour into breeding programmes to accelerate the development of drought tolerant groundnut genotypes.

Keywords: Groundnut, drought stress, SPAD chlorophyll reading, Water-use efficiency (WUE), Specific leaf area (SLA), number of stomata, stomatal conductance, generation mean analysis and gene action

Introduction
Groundnut (*Arachis hypogaea* L.) is a vital food and oilseed crop valued for its high oil (47-53%) and protein (25-36%) content. As a self-pollinated tetraploid (2n = 4x = 40) with a unique geocarpic fruiting habit, it adapts well to diverse agro-ecological regions and serves as a major source of edible oil, industrial raw material and nutrient-rich fodder. Its short duration and broad adaptability also support rapid crop improvement and multi-season cultivation. Globally, groundnut ranks as the fifth most cultivated oilseed crop, covering 27.9 million hectares with an annual production of 47 million tonnes.

India is the second-largest producer, with Gujarat, Rajasthan, Madhya Pradesh, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra contributing significantly. Gujarat is leading state in area (169.53 lakh ha) followed by Rajasthan (89.06), during the year 2023-24 (Ministry of Agriculture and Farmers Welfare, Govt. of India). Global groundnut agriculture covers 4.96 million hectares and produces 10.03 million tons with a productivity of 1616 kg/ha (FAOSTAT, 2023) [3]. However, nearly 80% of the crop is grown under rainfed conditions, making productivity highly vulnerable to erratic rainfall. With global vegetable oil demand expected to double by 2040, improving groundnut performance under water limited environments is increasingly important.

Drought is the most severe abiotic constraint reducing groundnut productivity, adversely affecting physiological processes such as photosynthesis, stomatal regulation, transpiration efficiency and water relations. These disruptions ultimately impair flowering, peg penetration and pod filling. Conventional selection for yield under drought is often unreliable due to strong genotype × environment interactions. Therefore, a deeper understanding of the physiological mechanisms governing drought tolerance is essential for developing resilient cultivars. Multi-generational evaluation provides a powerful approach to understand trait stability and the genetic control of key physiological responses under moisture stress. The present study focuses on physiological determinants of drought tolerance across generations to elucidate adaptive responses and identify traits with potential for use in breeding water-efficient, drought-resilient groundnut genotypes.

Materials and Methods

The present investigation was conducted at the field of AICRP on summer groundnut, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (MS) during *Kharif*-2023, *Summer*-2024 and *Kharif*-2024. Six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) were developed from cross TAG-24 × ICG 4670 for drought tolerance and pod yield. The experimental material consisted drought tolerant parent (ICG 4670) as donor and recipient parent (TAG-24) is high yielding and wider adaptability of groundnut genotypes. The cross TAG-24 × ICG 4670 was effected in *Kharif*-2023 to produce the F_1 seeds and breeding material like F_1, F_2, BC_1 and BC_2 and advancement was attempted in *Summer*-2024. During *Kharif*-2024, final evaluation trial conducted for study of inheritance of drought tolerance related traits and generation mean analysis, for physiological traits under both irrigated and drought (rainout shelter) condition. The characters *viz.*, SPAD chlorophyll meter reading, water-use efficiency (WUE), specific leaf area (SLA), number of stomata and stomatal conductance were analysed for this study.

Each character was analysed separately and the ANOVA was constructed as per Panse and Sukhatme (1995) [13]. The data was analysed for mean performance of the generations. Gene action has been worked by generation mean analysis as per six parameter model given by (Hayman, 1958) [4]. Adequacy of additive dominance effect was detected by individual scaling test; three tests of scale were carried out to detect presence or absence of gene interaction by using formulae given by Mather (1949) [10] and Hayman and Mather (1957) [5]. Cavalli's (1952) [2] joint scaling test was applied to test the adequacy of additive dominance model. Whenever, the model was found inadequate, Hayman (1958) [4] six parameter model was used to estimate the different gene effects. Statistical analysis of data was done by using software package INDOSTAT.

Results and Discussion

Analysis of Variances

The analysis of variance for five characters is presented in Table 1 for two crosses under both irrigated and drought (rainout shelter) condition.

Under irrigated condition (Table 1), the analysis of variance revealed significant differences among treatments in cross TAG-24 × ICG 4670 i.e., parents and their derived generations (F_1, F_2, BC_1 and BC_2) for majority of traits. This indicates the presence of substantial genetic variability among the parents and their progenies. However, trait such as water use efficiency exhibited non-significant differences, suggesting limited genetic variation for this character.

Under drought condition (Table 1), the analysis of variance showed significant differences among treatments in cross TAG-24 × ICG 4670 i.e., parents and their respective generations (F_1, F_2, BC_1 and BC_2) for all traits studied the, indicating considerable genetic variability under moisture stress.

Mean performance of parents and generations

The mean performance of parents and their crosses for physilogical traits in groundnut are presented in Table 2.1 and 2.2 for irrigated and drought condition respectively. Higher values are desirable for all traits under study except for days to specific leaf area for which lower values are preferred. The trait wise results are discussed as below:

Cross: TAG-24 × ICG 4670

SPAD chlorophyll meter reading

Under irrigated condition, SPAD chlorophyll meter readings ranged from (45.44) to (49.36) across the parental genotypes and their derived generations. The BC_2 generation recorded the highest chlorophyll content (47.39), followed by F_1 (46.67), F_2 (46.54) and BC_1 (46.19). Among the parents, TAG-24 exhibited a SPAD value of (45.44), whereas ICG 4670 had a slightly higher reading of (49.36). The elevated SPAD values in BC_2 and F_1 indicate enhanced chlorophyll retention and potentially higher photosynthetic efficiency under optimal conditions.

Under drought condition, SPAD chlorophyll meter readings declined across all generations except P_2, with values ranging from 37.10 to 49.10. The BC_2 generation recorded the highest SPAD value (45.98), followed by F_2 (44.59), F_1 (42.71) and BC_1 (38.55). Among the parents, TAG-24 and ICG 4670 recorded (37.10) and (49.10) respectively. The reduction in SPAD readings under drought reflects a decrease in chlorophyll content due to stress induced degradation. However, the relatively higher values in BC_2 and F_2 suggest better chlorophyll retention and photosynthetic resilience highlighting their potential for use in drought tolerant breeding programs.

Water use efficiency (µmoles $CO_2/m^2/s$)

Under irrigated conditions, water use efficiency (WUE) among the parental genotypes and derived generations varied from (4.29) to (6.62). The highest WUE was observed in F_2 (5.40), followed by F_1 (5.13), BC_2 (5.13) and BC_1 (4.84). The parental lines TAG-24 and ICG 4670 recorded WUE values of (4.29) and (6.62) respectively.

Under drought stress, water use efficiency (WUE) exhibited noticeable variation across generations, ranging from (2.56) to (6.17). The highest WUE was recorded in the BC_2 generation (6.17), followed by F_2 (4.65), F_1 (4.24) and BC_1 (3.74). Among the parental genotypes, TAG-24 and ICG 4670 showed WUE values of (2.56) and (6.02) respectively.

Specific leaf area (cm^2/g)

Under irrigated condition, specific leaf area (SLA) values ranged from (168.73) to (197.83) cm^2/g across the parental lines and their derived generations. The BC_1 generation exhibited the highest SLA (191.96 cm^2/g), followed closely by F_1 (190.00 cm^2/g), F_2 (185.11 cm^2/g) and BC_2 (185.05 cm^2/g). Among the parents, TAG-24 recorded a considerably higher SLA (197.83 cm^2/g) compared to ICG 4670 (168.73 cm^2/g). Higher SLA under irrigated conditions is generally associated with thinner leaves and rapid growth contributing to increased light interception and photosynthetic surface area. The elevated SLA in BC_1 and F_1 indicates vigorous vegetative development, which could be advantageous for biomass accumulation under optimal moisture conditions.

Under drought stress, specific leaf area (SLA) values increased across all generations and parental lines except BC_2, ranging from (170.34) to (214.97 cm^2/g). The BC_2 generation exhibited the lowest SLA (170.34 cm^2/g), followed by F_2 (191.37 cm^2/g) indicating thicker and more compact leaves as a typical adaptive trait under water stress. In contrast, BC_1 (202.02 cm^2/g) and F_1 (194.37 cm^2/g) maintained relatively higher SLA values suggesting a less conservative water-use strategy. Among the parents, TAG-24 recorded an SLA of (241.97 cm^2/g), whereas ICG 4670 showed a lower value of (173.31 cm^2/g). The reduction in SLA under drought is indicative of structural adjustments aimed at reducing transpirational water loss and enhancing drought tolerance. Generations like BC_2 and F_2 with lower SLA values may possess better physiological resilience under limited water availability.

Number of stomata

Under irrigated condition, the number of stomata varied from (12.91) to (20.36) among the different generations and parental lines. The BC_2 generation recorded the highest stomatal density (17.68), followed by F_2 (17.40), F_1 (16.38) and BC_1 (13.81). Among the parents, ICG 4670 exhibited a higher number of stomata (20.36) compared to TAG-24 (12.91). A greater number of stomata under irrigated conditions reflects the enhanced gas exchange capacity, facilitating efficient photosynthesis and transpiration. The elevated stomatal numbers in BC_2 and F_2 suggest their potential for high photosynthetic efficiency and productivity when water is not a limiting factor.

Under drought stress, the number of stomata showed a increase across generations, ranging from (14.46) to (20.51). The BC_2 generation maintained the highest stomatal count (19.86), followed by F_2 (19.70), F_1 (16.58) and BC_1 (15.73). Among the parents, ICG 4670 had a higher stomatal number (20.51) than TAG-24 (14.46). The relatively higher stomatal numbers retained in BC_2 and F_2 imply a balanced strategy, possibly enabling sustained gas exchange and moderate water conservation, thereby contributing to their better performance under moisture deficit conditions.

Stomatal Conductance (μmoles/m^2/s)

Under irrigated condition, stomatal conductance among the parental lines and their respective generations ranged from (0.249) to (0.587 μmoles/m^2/s). The BC_2 generation exhibited the highest conductance (0.453 μmoles/m^2/s), followed closely by F_1 (0.414), F_2 (0.345) and BC_1 (0.317). Among the parents, ICG 4670 recorded a conductance of (0.587 μmoles/m^2/s), while TAG-24 had the lowest value (0.249 μmoles/m^2/s). Higher stomatal conductance under well-watered conditions indicates efficient gas exchange and transpiration, supporting optimal photosynthesis and growth. Generations such as BC_2 and F_1 with elevated stomatal conductance reflect superior physiological performance under non stress condition and are potential candidates for maximizing productivity.

Under drought condition, stomatal conductance values increased across all generations except P_1, ranging from (0.233) to (0.640 μmoles/m^2/s). The BC_2 generation maintained the highest conductance (0.529 μmoles/m^2/s), followed by F_1 (0.418), F_2 (0.416) and BC_1 (0.328). Among the parents, ICG 4670 showed a higher value (0.640 μmoles/m^2/s) compared to TAG-24 (0.233 μmoles/m^2/s). Reduction in stomatal conductance of P_1 reflects a typical drought induced response aimed at reducing water loss through transpiration. Notably, the ability of BC_2 and F_1 to sustain relatively higher conductance even under moisture stress suggests enhanced stomatal regulation and water use dynamics making them promising for drought tolerance breeding programs.

Estimates of scaling tests for detecting non-allelic interactions

The results on scaling test (A, B, C and D) in respect of physiological and drought tolerance related traits have been tabulated in (Table 3) under irrigated and drought stress (rainout shelter) condition. The result for cross: TAG-24 × ICG 4670 is mentioned under two conditions as follow:

Under irrigated condition (Table 3)

SPAD chlorophyll meter reading

For SPAD chlorophyll meter reading trait, all joint scaling tests were found non-significant showing additive dominance model was adequate to explain all the genetic variations.

Water use efficiency

For water use efficiency, scaling test 'B' (-1.48) was negatively significant while scaling test 'D' (0.83) was found positively significant. The joint scaling test was found highly significant for water use efficiency in the cross TAG-24 × ICG 4670 which indicates inadequacy of additive dominance model to explain all the genetic variation.

Specific leaf area

All joint scaling tests were found non-significant showing additive dominance model was adequate to explain all the genetic variations as the chi-square values of both the crosses were non-significant.

Number of stomata
In case of number of stomata, the scaling test 'B' (3.64) and 'D' (3.31) was positively significant. Joint scaling test was found highly significant which indicates the presence of epistasis.

Stomatal Conductance
Scaling test 'B' (-0.09), 'C' (-0.28) and 'D' (-0.08) was negatively significant in the cross TAG-24 × ICG 4670 for stomatal conductance. The joint scaling test for stomatal conductance was found significant, indicating inadequacy of additive dominance model to explain all the genetic variation.

Under drought condition (Table 3)
SPAD chlorophyll meter reading
For SPAD chlorophyll meter reading, scaling tests 'C' (6.74) and 'D' (4.46) were significant, which indicates additive dominance model was inadequate to explain all the genetic variations.

Water use efficiency
For water use efficiency, scaling tests 'A' (0.67), 'B' (2.27) and 'C' (1.53) were positively significant and 'D' (-0.70) was negatively significant, showing additive dominance model was inadequate to explain all the genetic variations as the chi-square values of both the crosses were significant.

Specific leaf area
In case of specific leaf area, scaling tests 'A' (-32.31) and 'B' (-27.00) were negatively significant, showing additive dominance model was inadequate to explain all the genetic variations as the chi-square values of both the crosses were significant.

Number of stomata
For number of stomata, scaling test 'B' (2.63), 'C' (10.65) and 'D' (3.79) were found positively significant, which indicates inadequacy of additive dominance model to explain all the genetic variation.

Stomatal Conductance
Scaling tests 'C' (-0.042) and 'D' (-0.023) was negatively significant. The joint scaling test for stomatal conductance was found significant, indicating inadequacy of additive dominance model to explain all the genetic variation.

Estimates of gene effects for yield and yield contributing characters
The estimates of m (mean), major genetic effects additive [d] and dominance [h] and non-allelic gene interactions (i, j and l) based on six parameter model (Hayman, 1958) [4] for physiological traits for cross: TAG-24 × ICG 4670 (Table 4 and Table 5) under both irrigated and drought stress condition respectively. The parameter [m] was significant for all the characters under study.

Cross: TAG-24 × ICG 4670
The gene effects estimated by using perfect fit model in respect of traits associated with pod yield in groundnut has been presented in Table 4 and Table 5 for irrigated and drought condition respectively.

SPAD chlorophyll meter reading
Under irrigated condition, from estimates of genetic parameters, it was observed that significant mean (m) (46.39) effect was observed, but additive (d) and dominance (h) effects were non-significant, indicating an absence of non-allelic interactions for this trait.
Under drought stress, from estimates of genetics parameters, it was observed that 'd' (-7.43) and 'h' (-9.68) was negatively significant. The interaction component 'i' (-9.28) was observed negatively significant, while 'l' (11.82) was estimated positively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', revealing epistasis was predominantly of duplicate type for SPAD chlorophyll meter reading (SCMR). The duplicate type of epistasis was earlier noticed by Upadhyaya *et al.*, (2011) [17] and Lal *et al.*, (2005) [8] reported preponderance of additive gene action in the inheritance of SCMR.

Water use efficiency
Under irrigated condition, from estimates of genetic parameters, it was observed that 'h' (-2.01) was negatively significant. The interaction component 'i' (-1.67) was observed negatively significant, while 'l' (2.90) was estimated positively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', revealing epistasis was predominantly of duplicate type for water use efficiency.
Under drought stress, from estimates of genetic parameters, it was observed that 'd' (-2.52) was negatively significant and 'h' (1.36) was positively significant. The interaction component 'i' (1.41) was observed positively significant, while 'l' (-4.36) was estimated negatively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', with presence of duplicate epistasis for water use efficiency. Interestingly, John *et al.*, (2012) [7] has found significant role of non-additive gene actions for this trait.

Specific leaf area
Under irrigated condition, significant mean (m) (169.69) effect with high magnitude were observed. Additive (d) (14.54) effect was positively significant, but non-allelic interactions were absent, suggesting the preponderance of additive gene effects for specific leaf area.
Under drought condition, from estimates of genetic parameters, it was observed that 'd' (31.67) was positively significant and 'h' (-34.03) was negatively significant. The interaction component 'l' (80.07) was estimated positively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', revealing epistasis was predominantly of duplicate type for specific leaf area. John *et al.*, (2012) [7] found predominant role of non-additive gene actions for this trait which is parallel with present finding. Meanwhile, based upon generation mean analysis Suriharn *et al.*, (2005) [16] and Nigam *et al.*, (2001) [12] reported predominance of additive gene effect in the control of SLA in groundnut. Similar findings were noticed by Jayalakshmi *et al.*, (1999) [6] and Lal *et al.*, (2005) [8] in diallel analysis. Interestingly, role of additive and non-additive gene action for SLA were earlier reported by Venkateswaralu *et al.*, (2007) [18] and Upadhyaya *et al.*, (2011) [17].

Number of stomata

Under irrigated condition, from estimates of genetic parameters, it was observed that 'd' (-3.86) and 'h' (-6.85) were negatively significant. The interaction component 'i' (-6.63) was observed negatively significant, however 'l' (9.62) was estimated positively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', with presence of duplicate epistasis.

Under drought stress, from estimates of genetic parameters, it was observed that 'd' (-4.13) and 'h' (-8.50) were negatively significant. The interaction component 'i' (-7.59) was observed negatively significant, however 'l' (4.54) was estimated positively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', with presence of duplicate epistasis. Role of both additive and non-additive gene effects were significantly ascertained by John *et al.*, (2012) [7], Pitaloka *et al.*, (2022) [14]. While, Ramya *et al.*, (2021) [15] reported importance of non-additive gene action for this trait in wheat.

Stomatal Conductance

Under irrigated condition, from estimates of genetic parameters, it was observed that 'd' (-0.135) was negatively significant while 'h' (0.156) was positively significant. The interaction component, only 'i' (0.161) was observed positively significant, suggesting early selection benefits for this trait.

Under drought stress, from estimates of genetic parameters, it was observed that 'd' (-0.201) was negatively significant and 'h' (0.028) was positively significant. The interaction component only 'l' (-0.051) was estimated negatively significant. Opposite sign observed for genetic component dominance 'h' and dominance x dominance 'l', with presence of duplicate epistasis for stomatal conductance. John *et al.*, (2012) [7] reported predominance of both additive and nonadditive gene action for stomatal conductance. However, Ramya *et al.*, (2021) [15] reported importance of non-additive gene action for this trait in wheat.

Table 1: Analysis of variance (ANOVA) of six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) for physiological and drought tolerance related traits for cross: TAG-24 × ICG 4670 of groundnut under irrigated and drought condition.

Sr. No.	Name of characters	Cross: TAG-24 × ICG 4670			
		Irrigated Condition		Drought Condition	
		Mean sum of squares		Mean sum of squares	
		Generations	Generations	Generations	Error
	Degree of Freedom	5	5	5	10
1.	SPAD Chlorophyll meter reading (SCMR)	5.48**	1.74	62.01**	0.32
2.	Water use efficiency (µmoles $CO_2/m^2/s$) (WUE)	1.81	0.58	5.89**	0.29
3.	Specific leaf area (cm^2/g) (SLA)	292.13**	62.59	2007.58**	121.88
4.	Number of Stomata (NS)	22.03**	1.38	19.29**	0.58
5.	Stomatal Conductance (µmoles/m^2/s) (SC)	0.042**	0.002	0.062**	0.002
Note: *, ** significant at P = 0.05 and 0.01 per cent levels, respectively.					

Table 2.1: Mean performance of six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) for physiological and drought tolerance related traits under irrigated condition in Cross: TAG-24 × ICG 4670

Generations	P_1	P_2	F_1	F_2	BC_1	BC_2
SPAD Chlorophyll meter reading	45.44±1.75	49.36±1.90	46.67±1.79	46.54±0.77	46.19±0.79	47.39±0.85
Water use efficiency (µmoles $CO_2/m^2/s$) (WUE)	4.29±0.20	6.62±0.28	5.13±0.24	5.40±0.10	4.84±0.12	5.13±0.13
Specific leaf area (cm^2/g) (SLA)	197.83±7.78	168.73±6.58	190.00±7.34	185.11±3.12	191.96±3.45	185.05±3.38
Number of Stomata (NS)	12.91±0.59	20.36±0.79	16.38±0.65	17.40±029	13.81±0.26	17.68±0.33
Stomatal Conductance (µmoles/m^2/s) (SC)	0.249±0.009	0.587±0.024	0.414±0.016	0.345±0.007	0.317±0.006	0.453±0.011

Table 2.2: Mean performance of six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) for physiological and drought tolerance related traits under drought condition in Cross: TAG-24 × ICG 4670

Generations	P_1	P_2	F_1	F_2	BC_1	BC_2
SPAD Chlorophyll meter reading	37.10±1.46	49.10±1.89	42.71±1.64	44.59±0.73	38.55±0.66	45.98±0.82
Water use efficiency (µmoles $CO_2/m^2/s$) (WUE)	2.56±0.16	6.02±0.36	4.24±0.19	4.65±0.08	3.74±0.09	6.17±0.12
Specific leaf area (cm^2/g) (SLA)	241.97±10.68	173.31±7.03	194.37±7.75	191.37±3.19	202.02±3.78	170.34±3.05
Number of Stomata (NS)	14.46±0.57	20.51±0.81	16.58±0.66	19.70±0.32	15.73±0.28	19.86±0.36
Stomatal Conductance (µmoles/m^2/s) (SC)	0.233±0.009	0.640±0.028	0.418±0.021	0.416±0.009	0.328±0.006	0.529±0.010

Table 3: Estimation of scaling test and Chi square (χ2) test for physiological and drought tolerance related traits under irrigated and drought condition in Cross: TAG-24 × ICG 4670of groundnut

Characters	Cross: TAG-24 × ICG 4670									
	Irrigated condition					Drought Condition				
	A	B	C	D	χ^2	A	B	C	D	χ^2
SPAD Chlorophyll meter reading	0.28	-1.23	-1.96	-0.50	3.57	-2.70	0.16	6.74*	4.64**	7.91**
Water use efficiency	0.25	-1.48**	0.44	0.83**	21.07**	0.67*	2.27**	1.53*	-0.70**	27.19**
Specific leaf area	-3.90	11.37	-6.10	-6.79	2.87	-32.31*	-27.00*	-38.54	10.38	11.18*
Number of Stomata	-1.66	-1.32	3.64*	3.31**	21.79**	0.41	2.63*	10.65**	3.79**	34.47**
Stomatal Conductance	-0.02	-0.09**	-0.28**	-0.08**	33.98**	0.005	0.000	-0.042*	-0.023*	1.79*

Note: *, ** significant at P = 0.05 and 0.01 per cent levels, respectively.

Table 4: Estimation of gene effect for physiological and drought tolerance related traits under irrigated condition in Cross: TAG-24 × ICG 4670

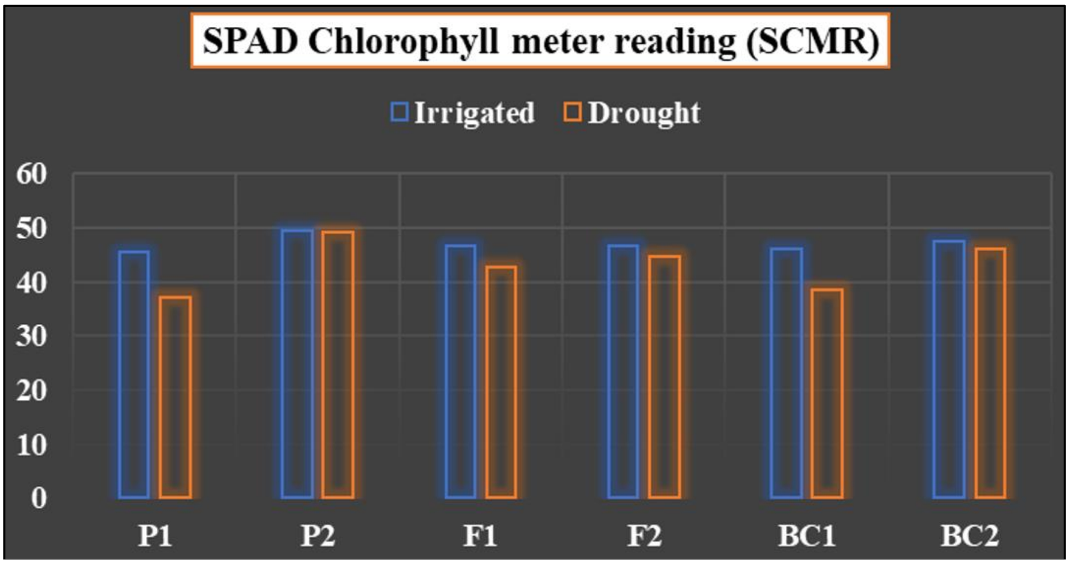
Characters	Genetic Parameters						Type of epistasis
	m	d	h	i	j	l	
SPAD Chlorophyll meter reading	46.39**	-1.96	0.34	--	--	--	--
Water use efficiency (μmoles $CO_2/m^2/s$) (WUE)	5.40**	-0.29	-2.01**	-1.67**	0.87	2.90**	Duplicate
Specific leaf area (cm^2/g) (SLA)	169.69**	14.54**	41.36	--	--	--	--
Number of Stomata (NS)	17.40**	-3.86**	-6.85**	-6.63**	-0.16	9.62**	Duplicate
Stomatal Conductance (μmoles/m^2/s) (SC)	0.345**	-0.135**	0.156**	0.161**	0.034	-0.038	---

Note: *, ** significant at P = 0.05 and 0.01 per cent levels, respectively.

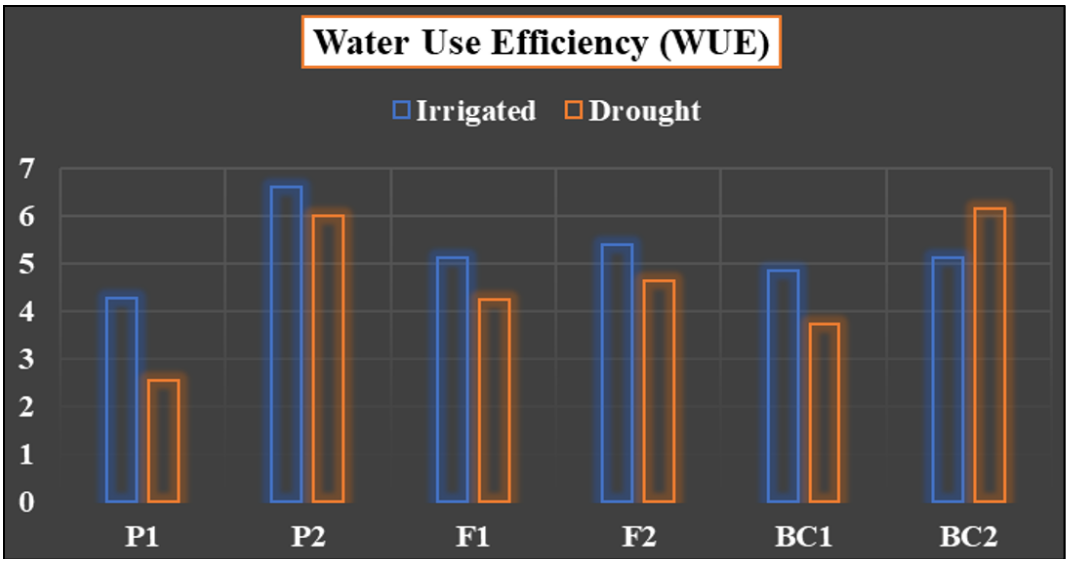
Table 5: Estimation of gene effect for physiological and drought tolerance related traits under drought condition in Cross: TAG-24 × ICG 4670

Characters	Genetic Parameters						Type of epistasis
	m	d	h	i	j	l	
SPAD Chlorophyll meter reading	44.59**	-7.43**	-9.68*	-9.28**	-1.43	11.82*	Duplicate
Water use efficiency (μmoles $CO_2/m^2/s$) (WUE)	4.65**	-2.52**	1.36**	1.41**	-0.79	-4.36**	Duplicate
Specific leaf area (cm^2/g) (SLA)	191.37**	31.67**	-34.03*	-20.76	-2.65	80.07**	Duplicate
Number of Stomata (NS)	19.70**	-4.13**	-8.50**	-7.59**	-1.11	4.54*	Duplicate
Stomatal Conductance (μmoles/m^2/s) (SC)	0.414**	-0.201**	0.028*	0.047	0.002	-0.051*	Duplicate

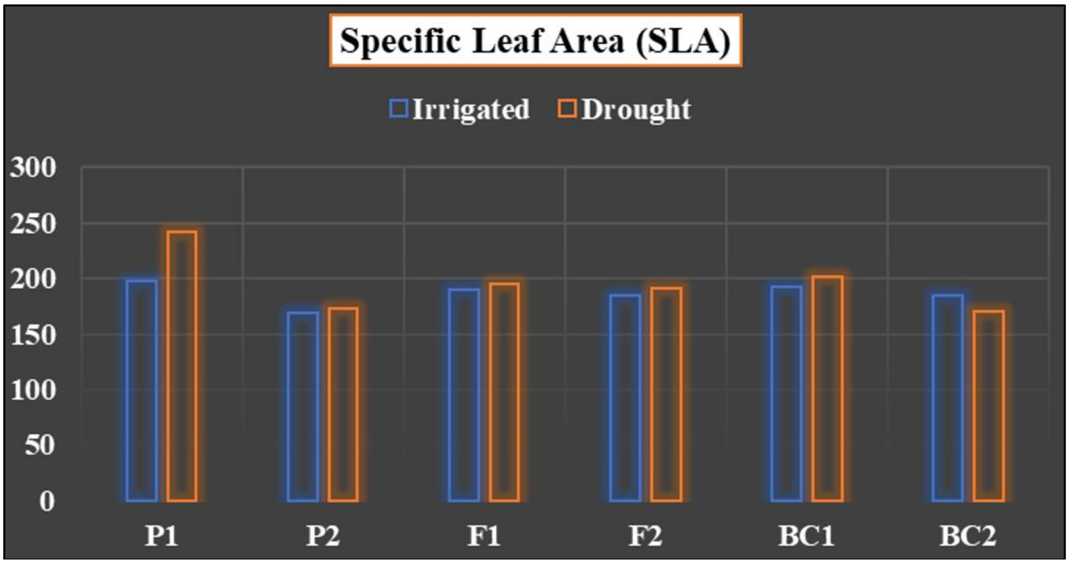
Note: *, ** significant at P = 0.05 and 0.01 per cent levels, respectively.



a) SPAD Chlorophyll meter reading

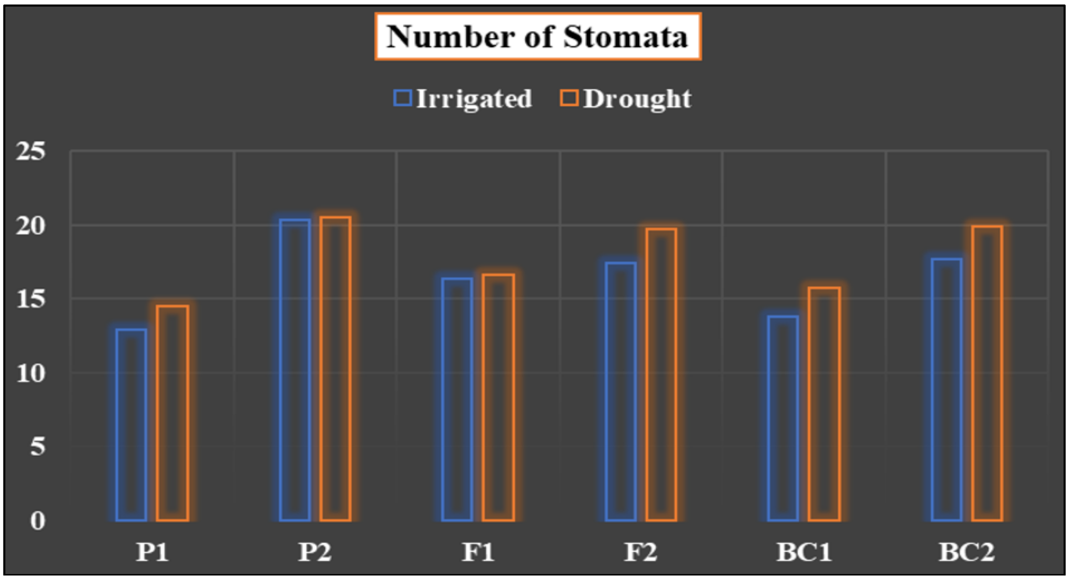


b) Water use efficiency

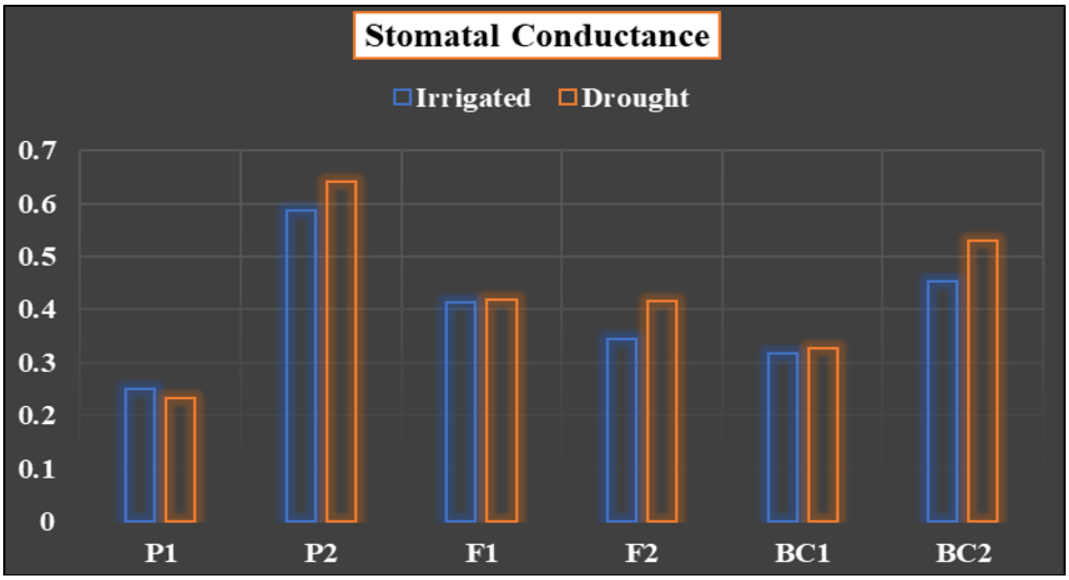


c) Specific leaf area

Fig 1: Comparison of morphological traits across generations under both irrigated and drought conditions



a) Number of stomata



b) Stomatal conductance

Fig 2: Comparison of morphological traits across generations under both irrigated and drought conditions

Conclusion

The present investigation demonstrated that drought stress significantly alters key physiological processes in groundnut, influencing chlorophyll stability, leaf structural efficiency and stomatal behaviour. The evaluation of five major physiological traits *viz.*, SPAD chlorophyll meter reading, water-use efficiency (WUE), specific leaf area (SLA), number of stomata and stomatal conductance, across six generations revealed substantial variability for drought-adaptive traits under both irrigated and stress environments. The distinct performance of parents and derived generations highlighted the contrasting physiological mechanisms underlying drought tolerance, with the drought-tolerant parent and BC_2 generation exhibiting superior SPAD values, higher WUE and favourable stomatal adjustment under moisture stress.

Generation mean analysis indicated that the additive dominance model alone could not sufficiently explain trait inheritance, necessitating a six-parameter model to capture the complexity of gene interactions. The predominance of dominance and dominance × dominance effects for SLA, stomatal conductance and WUE under drought stress suggests that non-additive gene action plays a crucial role in governing these traits. Such traits may be more effectively improved through hybridization followed by delayed selection. Conversely, significant additive and additive × additive effects observed for SPAD, WUE and stomatal number indicate the potential for early-generation selection when stable inheritance is present.

Overall, the findings underscore the value of integrating physiological indicators into drought-breeding pipelines. Emphasizing traits such as SPAD chlorophyll meter reading, WUE and stomatal behaviour can enhance the precision of selection and accelerate the development of groundnut genotypes with improved water-use efficiency and resilience to moisture stress.

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