



Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.367>

CORRELATION STUDIES AND ADAPTATION MECHANISMS OF AGRONOMIC AND PHYSIOLOGICAL TRAITS IN SOYBEAN (*GLYCINE MAX L. MERRILL*) F₂ POPULATIONS UNDER IRRIGATED AND DROUGHT CONDITIONS

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(Date of Receiving : 01-11-2025; Date of acceptance : 08-01-2026)

ABSTRACT

Drought stress is a major constraint in soybean productivity, particularly under rainfed conditions. To dissect the genetic and physiological basis of drought tolerance, a study was conducted involving two inter-varietal crosses: NRC 37 × EC 333901, and KDS 1201 × EC 333901. Evaluations were performed on 240 F₂ plants per environment under irrigated and drought-stressed conditions. Fifteen agronomic, phenological, yield-related, and physiological traits, including relative leaf water content (RLWC), normalized difference vegetation index (NDVI), canopy temperature (CT) and harvest index (HI) were recorded. Correlation analysis revealed consistent and significant associations of grain yield with harvest index (HI) under both conditions, reflecting their central role in yield determination. Under drought stress, physiological traits such as RLWC, NDVI, CT, and delayed leaf senescence (DLS) showed enhanced significance, highlighting their utility as indirect selection criteria. Among both the crosses, KDS 1201 × EC 333901 exhibited superior drought adaptability, characterized by stable physiological responses and strong yield performance. These results demonstrate that integrating physiological markers with conventional yield components can enhance the efficiency of drought-resilient soybean breeding. The findings provide a robust framework for selecting superior genotypes and deploying EC 333901 as a donor for improving stress tolerance.

Keywords : Soybean, Drought stress, Trait correlation, F₂ populations, Relative leaf water content (RLWC), Canopy temperature (C.T.)

Introduction

Soybean (*Glycine max* L. Merrill) stands out as a globally significant leguminous crop, prized for its high protein (approximately 40%) and oil content (nearly 20%). It plays an essential role in global food systems, livestock feed, and industrial applications. Additionally, due to its ability to fix atmospheric nitrogen, soybean supports sustainable farming by enhancing soil fertility in crop rotations. Currently, it is cultivated on over 130 million hectares worldwide, with its demand rising steadily due to its nutritional and biofuel utility (Jung *et al.*, 2023). In India, the crop is predominantly grown during the *Kharif* season under rainfed conditions, making it particularly susceptible to inconsistent monsoon patterns (Yaraşır *et al.*, 2024).

Among abiotic stresses, drought - especially when it coincides with the reproductive phase- is one of the most damaging constraints affecting soybean productivity. It interferes with photosynthesis, delays flowering, impairs pod development, and reduces final seed yield. Under extreme water deficit, yield losses can exceed 70–80% (Kim *et al.*, 2023). As climate change intensifies, the drought episodes are becoming more frequent and severe, emphasizing the urgent need to develop drought-resilient soybean varieties.

To achieve this, a deeper understanding of trait interactions under different water regimes is crucial. Correlation studies are instrumental in revealing associations between key agronomic, physiological, and yield-related traits. These relationships can guide

indirect selection for complex traits like yield, which is often influenced by multiple factors and may exhibit low heritability under stress. Traits such as canopy temperature (CT), relative leaf water content (RLWC), and the normalized difference vegetation index (NDVI) have emerged as valuable indicators of plant performance under water-limited conditions (Kim *et al.*, 2023).

Comparing correlations under both well-watered (control) and drought scenarios allows researchers to identify traits that consistently contribute to yield stability across environments. This approach supports the development of selection indices and the identification of promising genotypes with robust performance under variable moisture conditions (Yavas *et al.*, 2024). In this context, the present investigation aimed to evaluate the pattern of trait associations across irrigated and drought-stressed conditions in two F₂ soybean crosses involving the drought-tolerant donor EC 333901, providing insights for drought-resilient breeding strategies.

Materials and Methods

The study was conducted during 2021–2025 at the Post Graduate Institute Research Farm, M.P.K.V., Rahuri. Two soybean crosses were developed: NRC 37 × EC 333901 and KDS 1201 × EC 333901 involving parents with contrasting drought tolerance traits. Hybridization was carried out during *Kharif* 2022, Summer 2023, and *Kharif* 2023. F₁ and F₂ generations

were advanced through selfing during subsequent seasons.

Experimental Design and Stress Imposition

The experimental material comprised F₂ populations derived from two soybean crosses: NRC 37 × EC 333901 and KDS 1201 × EC 333901 which were evaluated under summer season conditions to investigate the expression and association of drought-responsive traits. Each cross was evaluated under two conditions- irrigated (control): recommended irrigation schedule was followed and drought-stressed condition: only two irrigations were applied, the first for germination and the second 15 days after germination, after which water was withheld to impose drought stress to assess drought tolerance. A Randomized Block Design (RBD) with three replications was followed. For each cross condition combination, 40 F₂ plants were evaluated per replication, totaling 120 plants per cross per condition. Thus, across two crosses, 240 F₂ plants were evaluated in irrigated and 240 plants in drought-stressed environments, amounting to 480 individual plants in total. Each plot consisted of 5-meter-long rows with a spacing of 45 cm × 10 cm.

Traits Recorded: Data were recorded on total 15 traits.

Table 1 : List of morphological, phenological, yield-related, and physiological traits evaluated in soybean F₂ populations under irrigated and drought conditions.

Sr. No	Trait	Abbreviation	Unit	Category	Description / Method
1.	Plant height	PH	cm	Morphological	Measured from base to tip of main stem at maturity.
2.	Number of primary branches/plant	PB	No./plant	Morphological	Counted at maturity.
3.	Number of clusters/plant	NCP	No./plant	Morphological	Total clusters counted per plant at maturity.
4.	Number of pods/cluster	NPC	No./cluster	Morphological	Average number of pods per cluster.
5.	Number of seeds/plant	NSP	No./plant	Yield-related	Counted at maturity.
6.	Number of pods/plant	NPP	No./plant	Yield-related	Total pods per plant at maturity.
7.	Grain yield	GY	g/plant	Yield-related	Weight of seeds per plant at harvest.
8.	Harvest index	HI	%	Yield-related	HI = (GY / total above-ground biomass) × 100.
9.	Days to 50% flowering	DF	Days	Phenological	Days from sowing to 50% of plants flowering.
10.	Days to maturity	DM	Days	Phenological	Days from sowing to physiological maturity.
11.	Relative leaf water content	RLWC	%	Physiological	RLWC = (FW – DW)/(TW – DW) × 100 (Barrs and Weatherley, 1962).
12.	Normalized Difference Vegetation Index at R2	NDVI-R2	—	Physiological	Measured using GreenSeeker

13.	Normalized Difference Vegetation Index at R5	NDVI-R5	—	Physiological	Measured using GreenSeeker
14.	Canopy temperature	CT	°C	Physiological	Measured at mid-day using infrared thermometer.
15.	Delayed leaf senescence	DLS	1–5	Physiological	Visual stay-green scoring at maturity (Ye <i>et al.</i> , 2019).

Statistical Analysis

Correlation analysis was carried out using Pearson's correlation coefficients to assess relationships among traits under irrigated and drought-stressed conditions. Significance was tested at 5% and 1% probability levels. All analyses were performed using the software OPSTAT (Sheoran *et al.*, 1998)

Results and Discussion

The present study investigated correlation analysis among key agronomic, physiological, and phenological traits in two soybean crosses: (1) NRC 37 × EC 333901 (2) KDS 1201 × EC 333901 evaluated under irrigated (well watered control) and drought-stressed restricted irrigated conditions of Summer 2024. In total, 240 F₂ plants (40 plants per cross per replication × 3 replications) were evaluated, comprising 120 plants under irrigated and 120 plants under drought-stressed conditions. The findings reveal the complex interplay of yield-contributing traits under varying environmental stresses, with a distinct shift in trait relationships between the two conditions. The overall pattern and strength of trait interrelationships under irrigated and drought conditions across the two crosses are visually summarized through correlation heatmaps (Figures 1–2).

Irrigated Condition

Under irrigated conditions, grain yield exhibited significant positive correlations with harvest index and major yield-contributing traits across both crosses (Tables 1 and 3), indicating efficient assimilate partitioning under favorable moisture availability. Normalized Difference Vegetation Index (NDVI) measured at R2 and R5 stages showed moderate to strong positive associations with grain yield, reflecting canopy vigor and photosynthetic capacity.

In NRC 37 × EC 333901, grain yield showed positive correlations with plant height, pods per plant, and harvest index, indicating coordinated vegetative growth and reproductive efficiency (Table 1; Figure 1). In contrast, KDS 1201 × EC 333901 showed negative associations between plant height and grain yield, while maintaining strong positive correlations with harvest index (Table 3; Figure 2), suggesting the advantage of moderate plant stature and improved source–sink balance in medium-tall genotypes. The

contrasting association patterns between plant height and grain yield observed in the two crosses indicate differential genetic control of plant architecture and assimilate partitioning, highlighting the importance of cross-specific selection strategies under optimal moisture conditions.

These findings align with Jumrani & Bhatia (2019) and Crusiol *et al.* (2021), who reported strong associations of HI with soybean yield under irrigated conditions, while NDVI effectively captured early canopy vigor. Negative associations of PH with yield under certain backgrounds are consistent with reports by Hou *et al.* (2019), highlighting the role of optimal plant stature in lodging resistance and resource use efficiency.

Drought-Stressed Condition

Under restricted irrigation, physiological traits gained prominence in sustaining yield (Tables 2 and 4). Grain yield retained strong correlations with HI across both crosses, but Relative Leaf Water Content (RLWC), NDVI-R2, NDVI-R5, and Canopy Temperature (CT) were more strongly linked to drought adaptation. In KDS 1201 × EC 333901, superior physiological adaptability was evident, with RLWC, NDVI-R2/R5 and cooler CT strongly associated with yield. Delayed Leaf Senescence (DLS) showed positive associations with RLWC and negative correlations with CT, underscoring the role of “stay-green” genotypes in maintaining yield under stress. NRC 37 × EC 333901 displayed moderate physiological buffering, suggesting partial drought tolerance. The comparatively weaker but significant associations of physiological traits with grain yield in NRC 37 × EC 333901 suggest that drought response in this cross is largely governed by yield component compensation rather than strong physiological homeostasis.

These results corroborate the findings of Yan *et al.* (2020), who demonstrated that HI and RLWC are reliable predictors of soybean yield under both irrigated and drought conditions. Similarly, Jumrani & Bhatia (2019) emphasized the importance of RLWC and CT for sustaining productivity under water deficit, while Crusiol *et al.* (2021) and de Andrade Franco *et al.* (2021) highlighted NDVI, CT, and RLWC as robust predictors of yield stability under drought.

The physiological relevance of these associations observed under drought stress is further supported by earlier studies. For instance, cooler canopy temperatures have been associated with higher yield under water stress in wheat (Babar *et al.*, 2006; Bazzaz *et al.*, 2015), indicating improved plant water status and adaptive capacity under limited moisture availability. Similarly, normalized difference vegetation index (NDVI) has been shown to reliably predict stress responses and grain yield in soybean (Dai *et al.*, 2023) and is strongly associated with leaf water potential (Ramirez *et al.*, 2019). Moreover, the positive role of delayed leaf senescence in sustaining yield under drought has been well documented (Shi *et al.*, 2017), reinforcing the significance of these physiological traits in the present investigation.

The present study demonstrated that while yield-contributing traits and harvest index predominantly influenced grain yield under irrigated conditions, physiological traits such as relative leaf water content, normalized difference vegetation index, canopy temperature, and delayed leaf senescence played decisive roles under drought stress. Crosses involving EC 333901, particularly KDS 1201 × EC 333901, exhibited superior drought resilience through enhanced physiological stability and efficient assimilate partitioning. These findings underscore the importance of integrating physiological indicators with conventional yield attributes in soybean breeding programs. EC 333901 proved to be an effective donor for drought tolerance, especially through delayed wilting and improved plant water status. Collectively,

the results advocate a multi-trait selection strategy combining yield components and physiological indicators to accelerate the development of climate-resilient soybean cultivars with stable performance across environments. Future research should focus on validating these trait relationships across environments and identifying genomic regions associated with drought adaptation to facilitate marker-assisted selection.

Conclusion

The study demonstrated that harvest index predominantly contributed to grain yield under irrigated conditions, whereas physiological traits such as relative leaf water content (RLWC), normalized difference vegetation index (NDVI), canopy temperature (CT), and delayed leaf senescence (DLS) were decisive under drought stress. Crosses involving EC 333901, particularly KDS 1201 × EC 333901 exhibited enhanced drought resilience through superior physiological buffering. These findings underscore the importance of integrating physiological traits with conventional yield components in soybean breeding programs. EC 333901 proved to be an effective donor for drought tolerance, particularly through delayed wilting and improved water status. Future research should focus on validating these traits across environments and identifying associated QTLs to facilitate marker-assisted selection for climate-resilient soybean improvement. The differential behaviour of the two crosses further indicates that genetic background strongly modulates the expression and utility of physiological traits under drought stress.

Table 1 : Simple correlation among seed-related traits in F₂ population of cross NRC 37 × EC 333901 under irrigated condition (based on 120 plants, pooled across three replications).

	PH	PB	NCP	NPC	NPP	NSP	HGW	HI	DF	DPM	RLWC	DLS	NDVI-R2	NDVI-R5	CT	GY
PH	1	0.66**	0.79**	0.76**	0.78**	0.57*	-0.27	0.702**	0.12	0.37	-0.17	0.60*	0.27	-0.31	0.73**	0.59*
PB		1	0.491	0.39	0.76**	0.80**	-0.47	0.835**	-0.43	-0.18	-0.66**	0.59*	-0.10	-0.71**	0.54*	0.78**
NCP			1	0.74**	0.640**	0.43	-0.39	0.560*	-0.003	0.29	-0.15	0.39	0.17	-0.36	0.42	0.43
NPC				1	0.61*	0.33	-0.42	0.316	0.09	0.32	-0.07	0.38	0.21	-0.25	0.68**	0.25
NPP					1	0.66**	-0.61**	0.812**	-0.36	-0.22	-0.67**	0.70**	-0.20	-0.65**	0.78**	0.85**
NSP						1	-0.44	0.659**	-0.31	-0.12	-0.60*	0.40	-0.02	-0.64**	0.52	0.63*
HGW							1	-0.246	0.63*	0.61*	0.70**	-0.29	0.52*	0.58*	-0.61**	-0.40
HI								1	-0.25	-0.11	-0.60*	0.67**	-0.03	-0.68**	0.44	0.94**
DF									1	0.79**	0.86**	-0.17	0.75**	0.64**	-0.10	-0.34
DPM										1	0.78**	-0.15	0.73**	0.46	-0.11	-0.34
RLWC											1	-0.43	0.64**	0.82**	-0.40	-0.71**
DLS												1	0.15	-0.27	0.58	0.72**
NDVI-R2													1	0.399	-0.08	-0.16
NDVI-R5														1	-0.311	-0.70**
CT															1	0.54*
GY																1

Note: *, ** Significant at 5% and 1% level of significance, respectively.

Table 2 : Simple correlation among seed-related traits in F₂ population of cross NRC 37 × EC 333901 under Drought condition (based on 120 plants, pooled across three replications).

	PH	PB	NCP	NPC	NPP	NSP	HGW	HI	DF	DPM	RLWC	DLS	NDVI-R2	NDVI-R5	CT	GY
PH	1	0.30	0.43	-0.55*	0.46	0.69**	0.48	-0.31	0.10	0.2	-0.1	0.44	0.37	0.328	0.10	0.31
PB		1	0.87**	-0.55*	0.74**	0.60*	-0.35	-0.86**	-0.63**	-0.59*	-0.86**	0.66**	-0.30	-0.498	0.82**	-0.14
NCP			1	-0.70**	0.93**	0.67**	-0.29	-0.85**	-0.68**	-0.58*	-0.83**	0.78**	-0.39	-0.325	0.76**	-0.34
NPC				1	-0.64**	-0.73**	0.40	0.76**	0.64**	0.46	0.73**	-0.81**	0.36	0.006	-0.51*	0.55*
NPP					1	0.62*	-0.0	-0.72**	-0.58*	-0.43	-0.70**	0.78**	-0.28	-0.32	0.57*	-0.18
NSP						1	-0.08	-0.67**	-0.38	-0.33	-0.56*	0.65**	-0.03	0.11	0.54*	-0.18
HGW							1	0.56*	0.75**	0.85**	0.67**	-0.4	0.71**	0.42	-0.52*	0.92**
HI								1	-0.772**	0.74**	0.92**	-0.80**	0.40	0.43	-0.84**	0.64**
DF									1	0.90**	0.82**	-0.68**	0.61*	0.48	-0.81**	0.81**
DPM										1	0.79**	-0.54*	0.72**	0.45	-0.81**	0.84**
RLWC											1	-0.77**	0.58*	0.51*	-0.79**	0.72**
DLS												1	-0.33	-0.25	0.66**	-0.46
NDVI-R2													1	0.24	-0.45	0.71**
NDVI-R5														1	-0.47	0.36
CT															1	-0.60*
GY																1

Note: *, ** Significant at 5% and 1% level of significance, respectively.

Table 3 : Simple correlation among seed-related traits in F₂ population of cross KDS 1201 × EC 333901 under irrigated condition (based on 120 plants, pooled across three replications).

	PH	PB	NCP	NPC	NPP	NSP	HGW	HI	DF	DPM	RLWC	DLS	NDVI-R2	NDVI-R5	CT	GY
PH	1	-0.77**	-0.52*	-0.30	0.78**	0.59*	0.81**	-0.83**	0.80**	0.86**	0.73**	-0.66**	0.50	0.63*	-0.58*	-0.83**
PB		1	0.69**	0.38	-0.47	-0.32	-0.61*	0.88**	-0.59*	-0.78**	-0.55*	0.72**	-0.15	-0.60*	0.79**	0.88**
NCP			1	0.75**	-0.14	0.19	-0.16	0.83**	-0.60*	-0.53*	-0.74**	0.86**	0.06	-0.5*	0.84**	0.83**
NPC				1	0.01	0.17	0.11	0.58*	-0.32	-0.41	-0.61*	0.78**	0.19	-0.59	0.58*	0.58*
NPP					1	0.76**	0.78**	-0.50	0.76**	0.74**	0.54*	-0.38	0.72**	0.50*	-0.30	-0.50*
NSP						1	0.65**	-0.22	0.37	0.47	0.07	-0.15	0.59*	0.20	-0.09	-0.22
HGW							1	-0.55*	0.66**	0.64**	0.37	-0.26	0.41	0.31	-0.29	-0.55*
HI								1	-0.73**	-0.77**	-0.74**	0.84**	-0.20	-0.63*	0.78**	0.98**
DF									1	0.79**	0.83**	-0.65**	0.42	0.51*	-0.60*	-0.73**
DPM										1	0.81**	-0.68**	0.43	0.73**	-0.67**	-0.77**
RLWC											1	-0.74**	0.32	0.72**	-0.67**	-0.74**
DLS												1	-0.104	-0.64**	0.84**	0.84**
NDVI-R2													1	0.43	-0.16	-0.20
NDVI-R5														1	-0.54*	-0.63*
CT															1	0.78**
GY																1

Note: *, ** Significant at 5% and 1% level of significance, respectively.

Table 4 : Simple correlation among seed-related traits in F₂ population of cross KDS 1201 × EC 333901 under drought condition (based on 120 plants, pooled across three replications).

	PH	PB	NCP	NPC	NPP	NSP	HGW	HI	DF	DPM	RLWC	DLS	NDVI-R2	NDVI-R5	CT	GY
PH	1	-0.02	0.14	0.58*	0.66**	0.51*	0.33	0.57*	0.12	0.22	-0.12	-0.09	0.29	0.20	-0.12	0.43
PB		1	0.82**	-0.41	-0.34	-0.51*	-0.48	-0.51*	-0.41	-0.57*	-0.52*	0.63**	-0.22	-0.40	0.69**	-0.56*
NCP			1	-0.15	-0.19	-0.33	-0.45	-0.46	-0.59*	-0.56**	-0.58*	0.72**	-0.24	-0.41	0.83**	-0.55*
NPC				1	0.81**	0.82**	0.83**	0.84**	0.39	0.69**	0.44	-0.58*	0.68**	0.68**	-0.42	0.81**
NPP					1	0.82**	0.83**	0.92**	0.63*	0.82**	0.60*	-0.67**	0.71**	0.69**	-0.58*	0.84**
NSP						1	0.84**	0.88**	0.40	0.75**	0.50*	-0.63*	0.61*	0.52*	-0.49	0.88**
HGW							1	0.90**	0.70	0.90**	0.76**	-0.85**	0.85**	0.76**	-0.72**	0.92**
HI								1	0.70	0.89**	0.67**	-0.81**	0.74*	0.74**	-0.74**	0.98**
DF									1	0.86**	0.87**	-0.88**	0.62*	0.7**	-0.89**	0.70**
DPM										1	0.91**	-0.94**	0.73**	0.84**	-0.84**	0.90**
RLWC											1	-0.91**	0.68*	0.79**	-0.83**	0.73**
DLS												1	-0.73**	-0.87**	0.92**	-0.86**
NDVI-R2													1	0.78**	-0.61*	0.75**
NDVI-R5														1	-0.73**	0.76**
CT															1	-0.77**
GY																1

Note: *, ** Significant at 5% and 1% level of significance, respectively.

Note- DF- Days to 50% flowering, DPM- Days to physiological maturity, PH- Plant height (cm), NCP-Number of clusters per plant, NPC-Number of pods per cluster, NPP- Number of pods per plant, PB-Number of primary branches per plant, HGW- Hundred grain weight (gm) and GY-Grain Yield per plant. RLWC: Relative leaf water content (%), NSP: Number of seeds/pod, HI: Harvest Index, DLS: Delayed leaf Senescence (1-5 scale), NDVI at R₂ : Normalized Difference Vegetation Index at R2 stage, NDVI at R₅: Normalized Difference Vegetation Index at R5 stage, CT: Canopy Temperature at grain filling stage

Correlation heatmaps of seed-related traits in NRC 37 × EC 333901

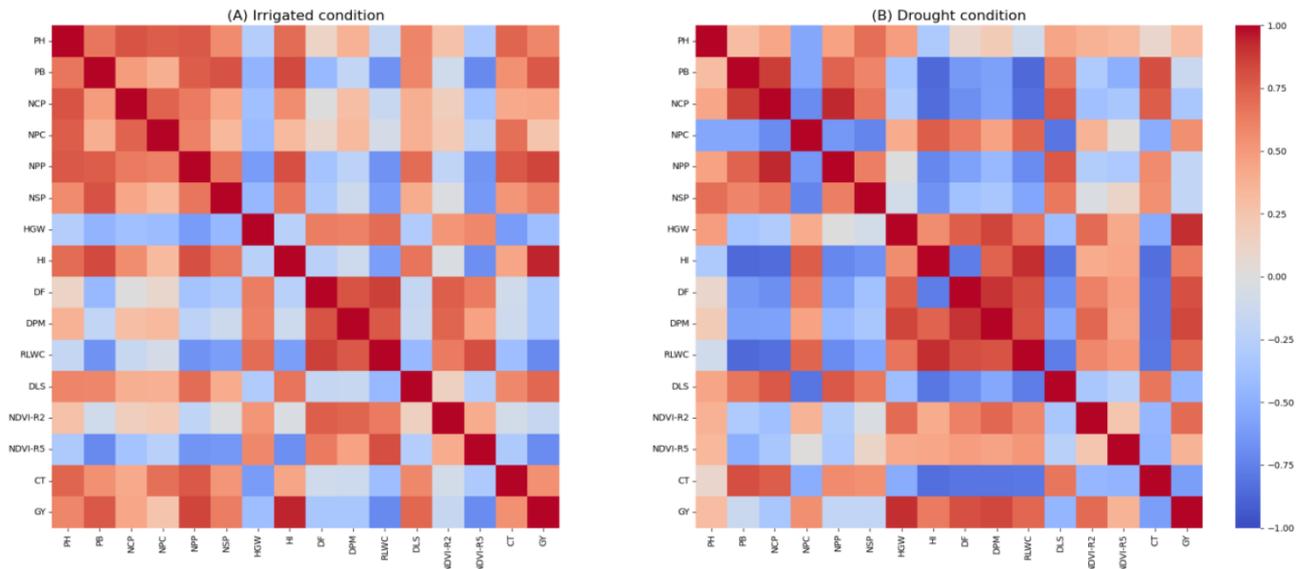


Fig. 1 : Correlation heatmap of seed-related traits under irrigated and drought conditions for cross NRC 37 × EC333901.

Correlation Heatmaps of Seed-related Traits in KDS 1201 × EC 333901

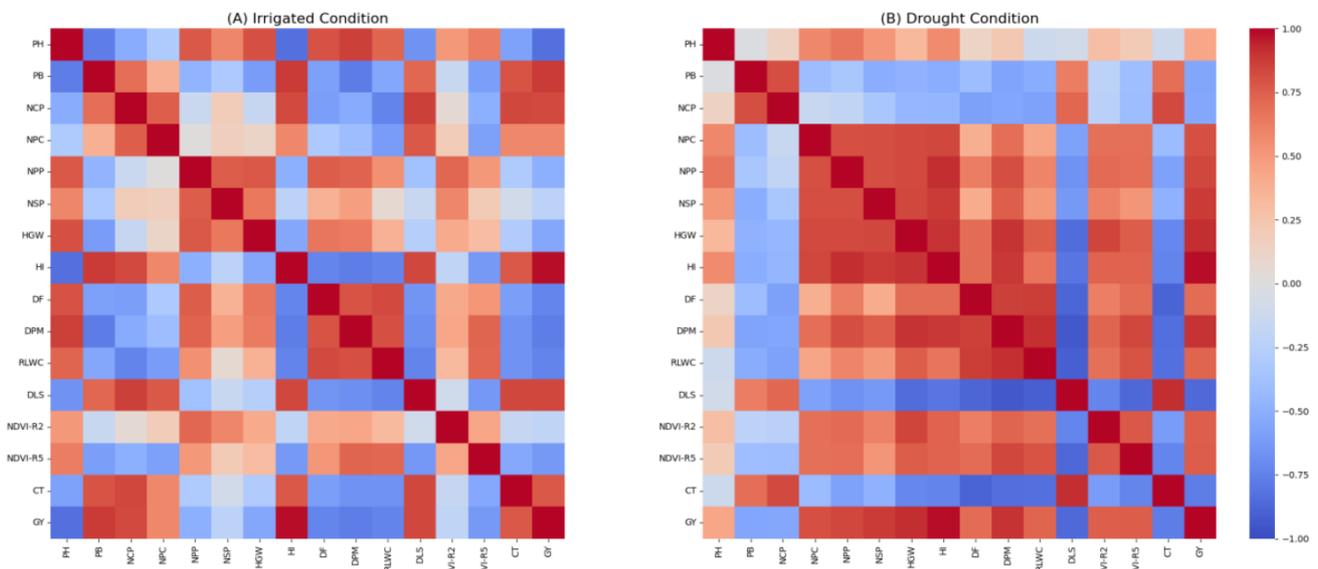


Fig. 2 : Correlation heatmap of seed-related traits under irrigated and drought conditions for cross KDS 1201 × EC333901.

Acknowledgement

The authors gratefully acknowledge the facilities provided by Department of Agricultural Botany, Post

Gradate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri-413722, Ahmednagar, Maharashtra, India.

Conflict of Interest

The authors declare no conflict of interest.

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