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Effects of Cadmium Stress on Various Growth and Yield Parameters of Soybean (*Glycine max* L.)

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ABSTRACT

Background: Cadmium (Cd) can reduce soybean growth and productivity, but responses may differ among cultivars. This study evaluated cultivar specific agronomic responses to increasing Cd stress using univariate and multivariate approaches.

Methods: A greenhouse pot experiment was conducted using a 3 × 4 factorial completely randomized design with three replications. Atlas 3616, Arisoy and Superb were exposed to 0, 2.5, 5.0 and 7.5 mg Cd kg^{-1}. Seven growth and yield traits were evaluated together with a composite tolerance index (CTI), principal component analysis (PCA) and Pearson correlations.

Result: Significant cultivar × Cd addition interactions occurred for all seven traits. At 7.5 mg kg^{-1}, seed yield declined by 24.32% in Atlas 3616, 36.65% in Arisoy and 43.79% in Superb. CTI ranked Atlas 3616 highest (83.67), followed by Arisoy (82.27) and Superb (80.40). The first three principal components explained 81.77% of total variation. Seed yield was positively associated with pod number (r= 0.659) and seed number (r = 0.613). Atlas 3616 showed the greatest overall agronomic trait retention under the highest Cd addition. However, because soil available and plant tissue Cd were not measured, these results cannot indicate Cd exclusion or food safety suitability.

Key words: Cadmium, Composite tolerance index, Genotype dependent, *Glycine max*, Growth traits, Principal component analysis, Seed yield.

INTRODUCTION

Cadmium (Cd) is a non essential and potentially toxic element that can accumulate in agricultural soils through industrial emissions, waste disposal, mining activities, atmospheric deposition and some agricultural inputs. Because Cd persists in soil and can enter the food chain through plant uptake, it is a concern for crop productivity and food quality. Cd exposure can disrupt nutrient homeostasis, photosynthesis, cellular redox balance and plant growth, although these effects vary with soil properties, species, genotype and exposure conditions (Antoniadis *et al.*, 2017; El-Rasafi *et al.*, 2022; Shafiullah *et al.*, 2025).

Soybean (*Glycine max* L.) an important global oilseed and protein crop utilized across food, feed and industrial sectors, is a typical short-day plant whose photoperiodic responses regarding flowering and maturity govern its latitudinal adaptability (Zhu *et al.,* 2024). Its seeds are rich in protein, oil, dietary fiber, minerals, vitamins and essential amino acids (Kudełka *et al.*, 2021; Alam *et al.*, 2023; Tondé *et al.*, 2026). Global soybean production reached approximately 420.87 million metric tons in 2024/25, while Türkiye produced about 180 thousand tonnes in 2024 (USDA-FAS, 2025; TÜİK, 2024). This importance makes cultivar response to Cd relevant to agronomic performance and food quality research.

Soybean can accumulate Cd from contaminated soils, but uptake, translocation and biological effects vary among genotypes and soil conditions. A global assessment of more than 5,000 soybean grain samples showed that soil pH and labile Cd fractions strongly influenced grain Cd accumulation (Zhang *et al.*, 2021). Screening of 25 soybean cultivars likewise revealed differences in seed Cd accumulation, translocation, plant height and seed biomass (Zhi *et al.*, 2020). Low Cd exposure may produce limited or stimulatory growth responses, whereas higher concentrations generally suppress growth and disturb physiological processes (Liu *et al.*, 2023). Therefore, maintenance of growth does not necessarily indicate low Cd accumulation in edible tissues. Evaluating vegetative and reproductive traits together is consequently more informative than ranking cultivars from a single trait.

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The soil used in the present experiment had a pH of 7.4; for soils within 7≤pH<8, a Cd reference value of approximately 1.5 mg kg^{-1} has been reported in Turkish soil quality literature (Çayır *et al*., 2025), providing context for the experimental Cd additions. This study aimed to determine the effects of cultivar, Cd addition and their interaction on seven growth and yield traits; compare overall trait retention using a study specific composite tolerance index and examine multivariate patterns and trait relationships using principal component and Pearson correlation analyses. Because Cd concentrations in plant tissues were not measured, cultivar comparisons were explained in terms of agronomic response rather than Cd exclusion or food safety suitability.

MATERIALS AND METHODS

Experimental design and Cd treatments

A greenhouse pot experiment was conducted at Düziçi, Osmaniye, Türkiye. Soybean seeds and $CdCl_2$, were obtained from the Department of Field Crops, Çukurova University, Adana, Türkiye. Thirty six pots (18 × 25 cm), each containing 7 kg of a 2:1 soil-peat mixture, were arranged in a 3 × 4 factorial completely randomized design with three replications. The factors were three soybean cultivars; Atlas 3616, Arisoy and Superb and four nominal Cd additions; 0, 2.5, 5.0 and 7.5 mg kg^{-1}. each cultivar × Cd addition combination was represented by three pots; one pot constituted one experimental unit. $CdCl_2$, was applied to the growth medium before sowing and 0 mg kg^{-1} served as the control. The initial soil pH was 7.4 and other soil properties are presented in Table 1. Twelve seeds were sown per pot on 22 July 2024 and thinned to six plants after emergence of the first fully developed trifoliate leaf. Plants within each pot were treated as subsamples and plant level measurements were averaged to obtain one observation per pot. Manual weeding was performed throughout the experiment and plants were harvested on 30 November 2024.

Data collection

At harvest, plant height (PH), first pod height (FPH), branch number per plant (BN), pod number per plant (PN), seed number per plant (SN), thousand-seed weight (TGW) and seed yield (Y) were recorded. All statistical analyses used pot level means.

Statistical analysis

Each trait was analyzed by two factor ANOVA for the 3 × 4 factorial design, with cultivar, Cd addition and their interaction as fixed effects and pot as the experimental unit. The model was:

$$Y_{ijk} = \mu + C_i + D_j + (C \times D)_{ij} + \varepsilon_{ijk}$$

where,

Y_{ijk} = Pot level observation.

μ = Overall mean.

C_i = Cultivar effect.

D_j= Cd addition effect.

$(C \times D)_{ij}$ = Cultiveter × Cd addition interaction.

ε_{ijk} = Residual error.

Degrees of freedom were 2, 3, 6, 24 and 35 for cultivar, Cd addition, interaction, residual error and total, respectively. When the interaction was significant, the 12 treatment combination means were compared using Fisher's LSD at p≤0.05. For each cultivar, percentage change relative to its control was calculated as:

$$\text{Per cent change} = \left[\frac{X_t - X_C}{X_C}\right] \times 100$$

Where,

X_t and X_C are the treatment and corresponding control means, respectively. Positive and negative values indicate increases and reductions.

Composite tolerance index

A study specific composite tolerance index (CTI) integrated retention of the seven traits at 7.5 mg Cd kg^{-1}. Trait retention was calculated as:

$$\text{Retention (\%)} = \left(\frac{X_{7.5}}{X_C}\right) \times 100$$

Values >100% were capped at 100 to prevent increases in one trait from disproportionately compensating for reductions in others. CTI was calculated as the arithmetic mean of the seven capped retention scores, with higher values indicating greater overall agronomic trait retention.

Multivariate analyses

Principal component analysis (PCA) was performed on all 36 pot level observations using the seven traits after centering and standardizing each variable to unit variance. PCA was based on the correlation matrix and eigenvalues, loadings, scores and individual and cumulative explained variance were obtained. PC1 and PC2 were used for the biplot, while the scree plot summarized explained variance. Pearson correlations among the seven traits were calculated using all 36 pot level observations, including controls. Two sided tests were used, with significance set at p<0.05. Statistical analyses and graphical preparation were performed using RStudio and Microsoft Excel.

RESULTS AND DISCUSSION

Effects of cultivar, Cd addition and their interaction

Factorial ANOVA showed significant cultivar × Cd addition interactions for all seven measured growth and yield traits (Table 2), demonstrating that soybean responses to Cd depended strongly on cultivar. Cd addition significantly affected all traits, whereas the cultivar main effect was significant for all traits except thousand-seed weight. Such genotype dependent responses are consistent with previous soybean studies showing substantial differences in growth and Cd tolerance among genotypes exposed to similar Cd conditions (Liu *et al.,* 2024; Ali *et al*., 2026).

Growth responses to Cd addition

Vegetative growth responded differently among cultivars (Table 3; Fig 1). At 7.5 mg Cd kg^{-1}, plant height (PH) decreased by 22.90% in Atlas 3616, 36.98% in Arisoy and 10.18% in Superb relative to their respective controls. Thus, Arisoy showed the highest height reduction, whereas Superb retained PH most effectively. Responses at lower Cd additions were less uniform. In Atlas 3616 PH declined by 6.78% at 2.5 mg kg^{-1}, partially recovered at 5.0 mg kg^{-1} (-3.62%) and then declined sharply at 7.5 mg kg^{-1}. Superb showed only a 0.93% reduction at 2.5 mg kg^{-1} and remained comparatively stable through 5.0 mg kg^{-1}. Such responses agree with Liu *et al*. (2023), who reported that relatively low Cd exposure may produce limited or stimulatory growth responses in soybean, whereas higher concentrations increasingly suppress growth. First pod height (FPH) also differed among cultivars. At the highest Cd addition, FPH declined by 25.16% in Atlas 3616 and 12.85% in Superb but increased by 8.37% in Arisoy. Branch number (BN) showed a similarly variable response: Atlas 3616 and Arisoy experienced moderate reductions at 7.5 mg kg^{-1}, whereas Superb declined by 25.91% after showing increased BN at the two lower Cd treatments. These contrasting patterns indicate that individual morphological traits do not provide a consistent ranking of cultivar performance under Cd stress.

Physiological studies have associated Cd induced growth inhibition in soybean with disturbances in photosynthesis, mineral homeostasis, chlorophyll status and antioxidant processes (Liu *et al*., 2023; Jia *et al*., 2025; Mukhtar *et al*., 2025). These mechanisms provide plausible biological context for the present growth responses;

Table 1: Physiochemical characteristics of soil used in pots.

Saturation	pH	Salinity	Lime	Organic matter	P_2O_5	K_2O	Ca	Mg	Fe	Cu	Mn	Zn
45%	7.4	%			ppm							
		0.02	1.93	1.44	20.00	72.50	2970	440	2.5	0.52	0.78	0.68

Table 2: Factorial ANOVA for soybean growth and yield traits.

Trait	Cultivar F	Cd addition F	Cultivar × Cd F
PH	310.40***	234.89***	38.57***
FPH	71.03***	15.36***	15.35***
BN	3.54*	7.71***	4.76**
PN	116.55***	17.73***	14.78***
SN	1105.81***	83.89***	225.18***
TGW	3.00 ns	9.33***	8.34***
Y	30.47***	48.79***	5.42**

df= 2, 3 and 6 for cultivar, Cd addition and their interaction, respectively; residual df= 24. *P<0.05, **P<0.01, ***P<0.001; ns: Not significant.

Table 3: Growth and yield traits of three soybean cultivars under different Cd additions.

Cultivar	Cd addition (mg kg^{-1})	Plant height (cm)	First pod height (cm)	Branch number $plant^{-1}$	Pod number	Seed number $plant^{-1}$	Thousand seed weight unit (g)	Seed yield (kg ha^{-1})
Atlas 3616	0	67.340f	10.677a	2.643abc	45.543a	63.087b	246.47abc	4918.1a
Atlas 3616	2.5	62.773g	9.657c	2.363cd	41.170b	60.933c	192.93f	4445.0bc
Atlas 3616	5	64.900fg	8.150ef	2.627abcd	38.907bc	69.833a	210.98ef	4362.3bc
Atlas 3616	7.5	51.920i	7.990f	2.267d	38.400c	63.853b	216.95de	3721.9de
Arisoy	0	88.410a	9.913bc	2.777ab	37.057cd	47.203f	257.01a	4783.8ab
Arisoy	2.5	80.957b	10.380ab	2.703abc	28.737g	46.270f	228.19bcde	3442.0ef
Arisoy	5	77.150c	10.023bc	2.590bcd	35.073de	52.083e	225.31cde	3574.3de
Arisoy	7.5	55.720h	10.743a	2.637abc	29.317g	51.667e	193.92f	3030.4f
Superb	0	79.163bc	8.873d	2.483bcd	32.483ef	57.430d	217.46de	4002.3cd
Superb	2.5	78.427bc	8.700de	2.970a	36.603cd	57.080d	247.13ab	3988.6cd
Superb	5	73.867d	8.737de	2.607bcd	28.303g	43.170g	235.44bcd	3834.8de
Superb	7.5	71.103e	7.733f	1.840e	32.273f	32.753h	215.60de	2249.7g
$LSD_{0.05}$	-	2.653	0.617	0.361	2.661	1.625	21.317	467.868

Means sharing a lowercase letter within a trait are not significantly different by Fisher's LSD test at P≤0.05.

however, such physiological variables and tissue Cd concentrations were not measured in this experiment and therefore cannot be directly inferred from the present results.

Yield and yield component responses

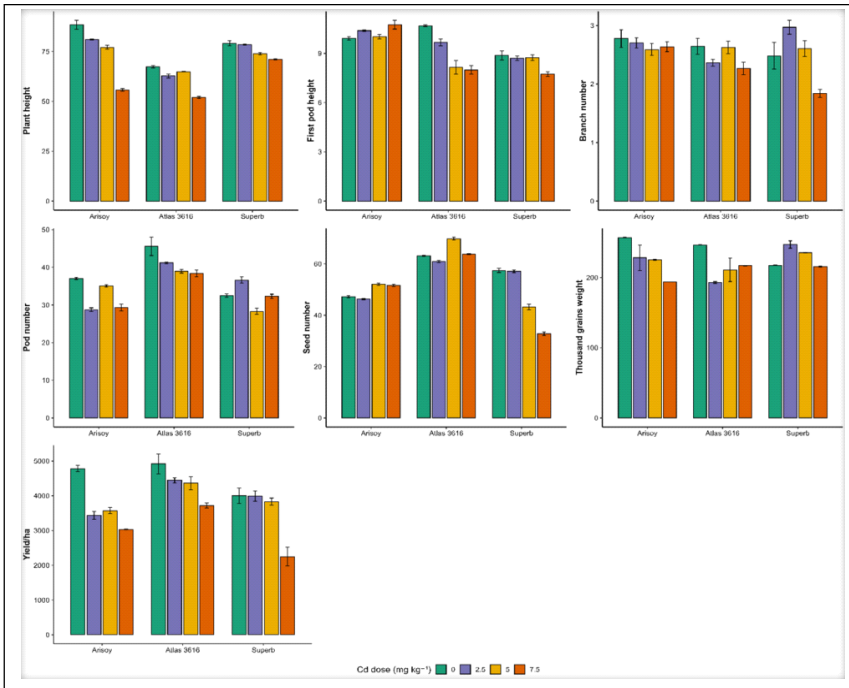
Reproductive traits and seed yield also showed significant cultivar × Cd addition interactions (Table 2 and 3; Fig 1). Pod number (PN) generally declined under Cd exposure, although the response was not consistently dose dependent. At 7.5 mg kg^{-1}, PN decreased by 15.68% in Atlas 3616 and 20.89% in Arisoy, whereas Superb remained close to its control (-0.65%). At lower treatments, the pattern was also non linear: Arisoy showed its greatest PN reduction at 2.5 mg kg^{-1}, while Superb increased PN at 2.5 mg kg^{-1} before declining at 5.0 mg kg^{-1}. Similar genotype dependent differences in reproductive performance have been reported among soybean accessions exposed to Cd (Ikhajiagbe *et al.*, 2021).

Seed number (SN) provided a clearer distinction among cultivars. Atlas 3616 maintained SN across treatments and showed a slight increase (+1.22%) at 7.5 mg kg^{-1}. Arisoy also maintained SN at the higher treatments, with a 9.46% increase at 7.5 mg kg^{-1}. In contrast, Superb showed marked reductions of 24.83% at 5.0 mg kg^{-1} and 42.97% at 7.5 mg kg^{-1}. TGW followed a different pattern: at 7.5 mg kg^{-1}, it decreased by 11.98% in Atlas 3616 and 24.55% in Arisoy but by only 0.85% in Superb. The contrasting responses of SN and TGW in Superb are particularly informative because they indicate that the major reproductive limitation at the highest Cd treatment was associated more strongly with the number of seeds produced than with individual seed mass.

Seed yield integrated these contrasting component responses. At 7.5 mg kg^{-1}, yield declined by 24.32% in Atlas 3616, 36.65% in Arisoy and 43.79% in Superb. Atlas 3616 therefore retained yield most effectively at the highest Cd addition. Arisoy showed substantial yield losses despite maintaining or increasing SN at the higher treatments. Superb performed comparatively well at 2.5 and 5.0 mg kg^{-1}, with yield reductions of only 0.34 and 4.19%, respectively, but became highly sensitive at 7.5 mg kg^{-1}. Previous studies have likewise demonstrated strong genotype dependence of soybean yield responses to Cd exposure (Ikhajiagbe *et al.*, 2021, 2022). Collectively, these results show that cultivar response cannot be judged reliably from a single vegetative or reproductive trait. In particular, the comparatively strong maintenance of PH and TGW in Superb did not translate into maintenance of SN or final yield under the highest Cd treatment.

Composite tolerance index

Because individual traits produced different cultivar rankings, the study specific composite tolerance index (CTI) was used to integrate retention of all seven traits at 7.5 mg Cd kg^{-1} (Table 4). Atlas 3616 had the highest CTI (83.67), followed by Arisoy (82.27) and Superb (80.40). The value of this multi trait assessment is illustrated particularly well by Superb: although it retained 89.82% of PH and 99.15% of TGW, retention of SN and yield fell to 57.03 and 56.21%, respectively. The CTI supports the conclusion that Atlas 3616 showed the greatest overall agronomic trait retention



Bars represent mean±SE (n= 3 independent pots).

Fig 1: Growth and yield responses of soybean cultivars to nominal Cd additions.

under the highest experimental Cd addition. The different trait patterns observed among the cultivars are consistent with reports that soybean genotypes may differ substantially in their response to Cd exposure (Liu *et al.,* 2024; Ali *et al.,* 2026).

Principal component analysis

The first three principal components together explained 81.77% of the total variance (Fig 2). PC1 accounted for 36.09%, PC2 for 29.72% and PC3 for 15.97%, while PC1, PC2 biplot represented 65.81% of the total variation. Seed yield, PN, SN and BN were oriented in a similar direction within the major multivariate space, indicating that variation in these reproductive and yield related traits contributed jointly to differentiation among cultivar × Cd-treatment observations.

In contrast, PH and TGW were separated from PN and SN along PC2, reinforcing the univariate observation that maintenance of vegetative stature or individual seed mass did not necessarily coincide with maintenance of reproductive output. FPH contributed most strongly to PC3. The scree plot further showed that inclusion of PC3 increased cumulative explained variance from 65.81 to 81.77%, after which the contribution of subsequent components was considerably smaller. The multivariate differentiation observed here is consistent with the use of PCA to integrate multiple Cd response traits in soybean germplasm screening (Ali *et al.*, 2026).

Pearson correlation analysis

Pearson correlation analysis based on all 36 pot level observations showed that seed yield was most strongly and positively associated with PN (r= 0.659, p<0.001) and SN (r = 0.613, p<0.001), followed by BN (r = 0.435, p<0.01) and TGW (r= 0.334, p= 0.046) (Fig 3). PN and SN were also positively correlated (r= 0.651, p<0.001). These relationships are consistent with reports that pod and seed number contribute strongly to soybean yield formation (Paraginski *et al.*, 2024; Zhang *et al.*, 2025). Earlier work has similarly emphasized seed number as an important determinant of soybean yield relative to individual seed size (Board, 1987). In the present experiment, the stronger association of yield with SN than with TGW supports this general pattern.

Additional relationships among non yield traits further demonstrated that growth and reproductive characteristics did not respond uniformly: PH was positively associated with TGW but negatively associated with SN, while FPH was positively associated with BN and BN with TGW (Fig 3). Because correlations were calculated using pooled observations across different cultivars and Cd treatments, they describe overall associations and should not be defined as cultivar specific causal relationships. Nevertheless, the correlation analysis complements the factorial and PCA results by showing that maintenance of reproductive components, particularly PN and SN, was closely associated with yield under the experimental conditions.

Environmental relevance and study limitations

The soil used in the experiment had an initial pH of 7.4. For soils within 7 ≤ pH < 8, Turkish soil quality literature reports a Cd reference value of approximately 1.5 mg kg^{-1} (Çayır *et al.*, 2025). Relative to this contextual benchmark, the nominal additions of 2.5, 5.0 and 7.5 mg Cd kg^{-1} were approximately 1.67, 3.33 and 5.00 fold higher, respectively and therefore represented progressively elevated experimental Cd stress. The experimental medium

Table 4: Relative trait retention and composite tolerance index of soybean cultivars at 7.5 mg Cd kg^{-1}.

Cultivar	PH	FPH	BN	PN	SN	TGW	Y	CTI	Rank
Atlas 3616	77.1	74.84	85.75	84.32	100	88.02	75.68	83.67	1
Arisoy	63.02	100	94.96	79.11	100	75.45	63.35	82.27	2
Superb	89.82	87.15	74.09	99.35	57.03	99.15	56.21	80.4	3

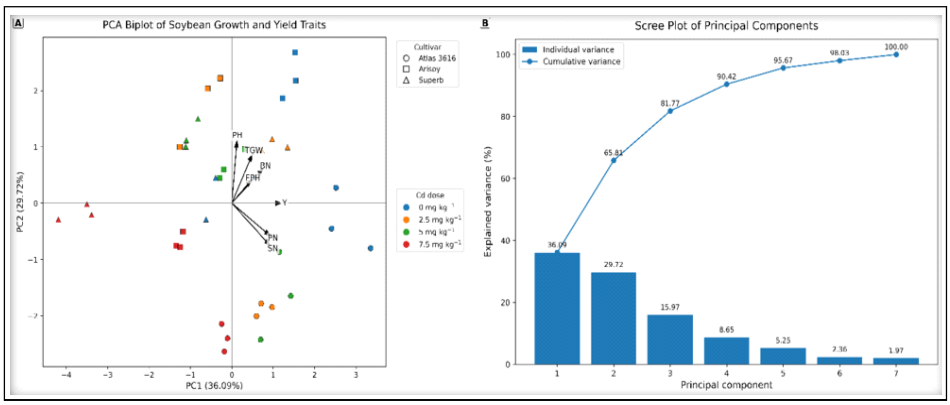


Fig 2: Principal component analysis of seven agronomic traits: (A) PC1-PC2 biplot and (B) scree plot showing individual and cumulative explained variance.

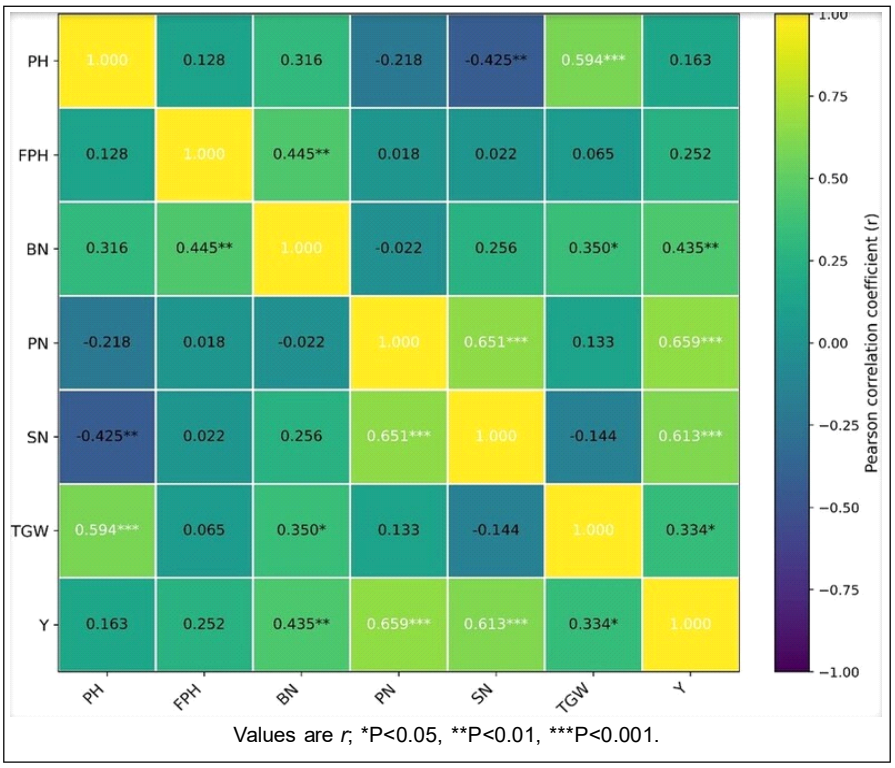


Fig 3: Pearson correlation matrix among seven agronomic traits based on 36 pot-level observations.

contained soil and peat, but baseline Cd and post treatment total or plant available Cd concentrations were not analytically determined. Soil properties can strongly influence Cd bioavailability and soybean grain accumulation, particularly through pH and labile Cd fractions (Zhang *et al.*, 2021). In addition, Cd concentrations were not measured in roots, shoots, pods, or seeds. This distinction is important because soybean genotypes can differ in Cd uptake and seed accumulation and acceptable growth performance does not necessarily indicate low Cd concentration in edible tissues (Zhi *et al.*, 2015, 2020). Consequently, the present data cannot determine whether a cultivar maintained growth through Cd exclusion, restricted translocation, tissue sequestration, or tolerance despite greater internal Cd accumulation. Future studies should combine agronomic performance with measurements of total and plant available soil Cd, tissue specific Cd concentrations and indices of Cd translocation and accumulation. Field validation will also be necessary before responses observed under greenhouse pot conditions can be translated into recommendations for Cd affected agricultural soils.

CONCLUSION

Soybean responses to Cd were strongly cultivar dependent, with significant cultivar × Cd addition interactions across all measured traits. At 7.5 mg Cd kg^{-1}, Atlas 3616 showed the greatest overall agronomic trait retention (CTI= 83.67), followed by Arisoy (82.27) and Superb (80.40). Although Superb maintained plant height and thousand-seed weight comparatively well, substantial reductions in seed number and yield highlighted the importance of reproductive traits in evaluating Cd response. PCA and correlation analyses further emphasized the contribution of pod and seed number to yield variation. Overall, Atlas 3616 performed best under the highest experimental Cd addition; however, because soil available and tissue Cd concentrations were not measured.

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Conflict of interest

The authors declare no conflicts of interest.

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