Phenotypic Variation and Correlation Analysis of Chickpea Germplasm under Heavy Metal Stress at Seedling Stage

Abstract

This study explores the phenotypic responses of diverse chickpea (*Cicer arietinum* L.) germplasms to heavy metal (Mn) stress, emphasizing seedling traits vital for plant establishment and growth. Chickpea seeds obtained from Acharya Narendra Deva University of Agriculture and Technology underwent meticulous examination and were stressed with manganese (Mn) supplementation at 200 µM Mn. Germination assays were meticulously conducted under controlled conditions, with data recorded over a 14-day span. Statistical analysis uncovered significant variations among germplasms concerning traits such as seed volume, germination percentage, 100-seed weight, speed of germination, field emergence, shoot and root lengths, seedling length, and vigour index-I. Correlation analysis delineated intricate interrelationships between these traits under heavy metal (Mn) stress. Notably, ICCV 92944 emerged as a superior variety, showcasing rapid germination, vigorous seedling growth, and remarkable stress tolerance. Other promising genotypes include HC 3, BG 267, and SBD 377. These findings offer valuable insights for breeding programs aimed at cultivating stress-tolerant chickpea varieties, thereby contributing to sustainable crop production in contaminated environments.

Introduction

The impact of high manganese (Mn) concentrations on plant physiology and nutrient dynamics is well-documented in the literature. Mn excess alters enzymatic activity and disrupts the uptake, redistribution, and utilization of essential nutrients such as calcium (Ca), iron (Fe), magnesium (Mg), nitrogen (N), and phosphorus (P) (Santos *et al.*, 2017; Li *et al.*, 2019). Studies have shown that *Mn* toxicity reduces leaf CO2 assimilation rate, stomatal conductance, and chlorophyll content (chlorophylls a and b) in various plant species (Stoyanova *et al.*, 2009; Santos *et al.*, 2017). Additionally, excessive Mn levels enhance the production of reactive oxygen species (ROS), leading to oxidative stress in plant cells and altering antioxidant enzyme activities (Santos *et al.*, 2017; Demirevska-Kepova*et al.*, 2004; Hannam and Ohki, 1988).

Differential tolerances to Mn toxicity have been observed among plant genotypes within species such as soybean (*Glycine max*), wheat (*Triticum aestivum*), bean (*Phaseolus vulgaris*), rice (*Oryza sativa*), and cowpea (*Vigna unguiculata*) (Foy et al., 1988). Mechanisms of Mn toxicity tolerance involve regulation of *Mn* uptake, translocation, and distribution, with specific transporters and detoxification genes identified in plant species like Arabidopsis and rice (Tsunemitsu *et al*., 2018; Li *et al*., 2019). Plants can mitigate Mn toxicity by limiting root absorption, retaining *Mn* in roots, or tolerating high Mn levels in shoots (Edwards and Asher, 1982; El-Jaoual and Cox, 1998; Blamey *et al*., 2015).

Chickpea (*Cicer arietinum* L.) is a globally significant pulse crop, yet its yield remains low and unstable, potentially due to soil acidity constraints (Maesen *et al*., 2007; Merga and Haji, 2019). Domestic cultivars lack diversity, and traditional breeding has not substantially improved productivity (Singh and Ocampo, 1997). Wild *Cicer* species offer potential for developing stress-tolerant cultivars, particularly against low pH stress (Berger *et al*., 2003). However, acid tolerance screening in chickpea has predominantly focused on aluminum toxicity, neglecting Mn toxicity (Hayes *et al*., 2012). Given the sensitivity of chickpeas to Mn toxicity and limited research in this area, screening for Mn toxicity tolerance is essential for cultivar improvement.

This study aimed to develop a screening protocol for evaluating chickpea tolerance to Mn toxicity, ranking wild *Cicer* accessions and chickpea germplasm accordingly. The research objectives included assessing various phenotypic traits under heavy metal stress conditions, such as seed volume, germination parameters, seed weight, shoot and root lengths, and seedling vigor indices. Identifying superior chickpea varieties resilient to heavy metal stress is critical for breeding programs focused on developing stress-tolerant cultivars, thereby ensuring sustainable crop production in contaminated environments.

Material and Methods

Seed Material Preparation: Chickpea (*Cicer arietinum* L.) seeds were sourced from a reputable agricultural research institution, specifically selected for their suitability for the study. The seeds were obtained from the experimental farm located atAcharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya. Prior to experimentation, the seeds underwent a thorough examination to ensure uniformity in size, shape, and maturity. Any damaged or immature seeds were discarded to maintain consistency in the seed material.

*Preparation of Heavy Metal Solutions:*The experiment included a specific treatment involving manganese (Mn) supplementation. Manganese was introduced into the solution in the form of manganese sulfate monohydrate (MnSO4·H2O). The concentration of manganese in the solution was set at 200 micromolar (µM Mn).

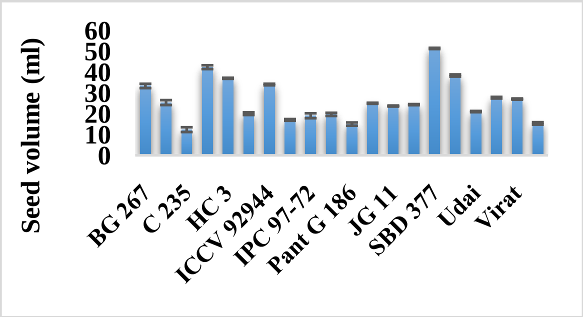
Germination Assay: The selected chickpea seeds were carefully cleaned and surface sterilized to remove any external contaminants. This was achieved by soaking the seeds in a 1% solution of sodium hypochlorite for 5 minutes, followed by thorough rinsing with deionized water to eliminate any residual disinfectant. Only intact, fully developed seeds were chosen for the germination experiment to ensure uniformity and reliability of results.Subsequently, the sterilized seeds were evenly distributed on germination paper (Standard Germination method as per ISTA regulation: Between Paper Method). Each Petri dish represented a different treatment group, including various concentrations of the Mn heavy metal solution (200 µM Mn) as well as a control group treated with deionized water. Three replicates of each treatment, consisting of 20 seeds per replicate, were prepared to account for variability and ensure robustness of the experimental data.The germination experiment was conducted in a controlled environment chamber set to mimic optimal growth conditions for chickpea. The chamber maintained a 16-hour light/8-hour dark cycle, with a constant temperature of 25°C during the experiment. Germination progress was monitored and recorded daily for a duration of 14 days. A seed was considered germinated when a radicle of at least 2 mm in length was visibly observed without the aid of magnification.This standardized germination assay allowed for the evaluation of chickpea seed germination under various heavy metal stress conditions, providing valuable insights into the potential effects of heavy metal contamination on chickpea seedling establishment and growth.

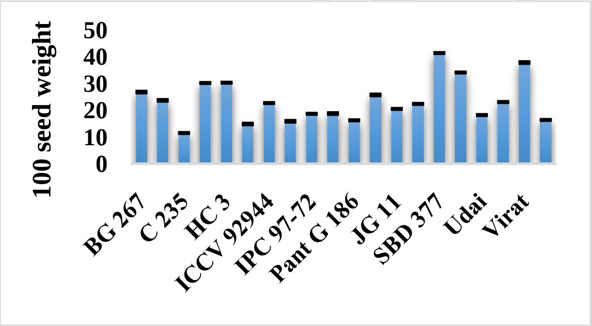
Statistical analysis: The statistical analysis was conducted using SPSS version 16. Tukey's test and correlation analysis were employed to examine the relationships between different variables. Tukey's test was applied to compare the means of multiple groups, while correlation analysis was used to assess the strength and direction of the relationships between pairs of variables. This statistical model allowed for a comprehensive examination of the data, providing insights into the significance of differences between groups and the associations among various traits.

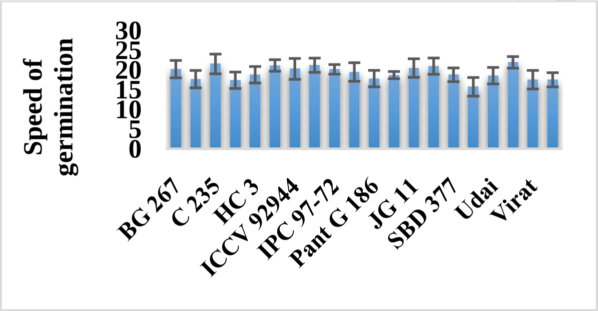
Results:

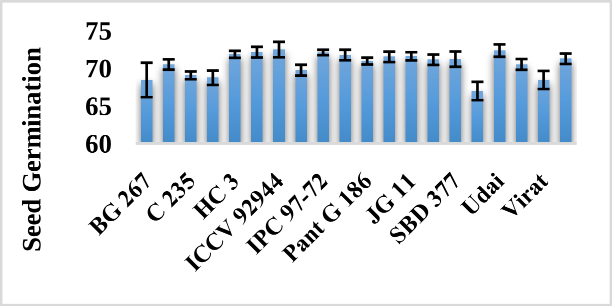
Mean values and standard deviations of various phenotypic traits observed in twenty chickpea germplasms subjected to heavy metals stress (Supplementary Table 1). Significant variations ($p < 0.05$) were noted among the germplasms for all traits evaluated, reflecting the diverse responses of chickpea genotypes to heavy metal stress conditions.Seed volume is a critical trait influencing seedling vigor and nutrient reserves. Among the studied germplasms, BG 267 exhibited the highest mean seed volume of 33.33 ± 1.04, indicating potential for robust seedling establishment even under heavy metals stress. Similarly, SBD 377 and JAKI 9218 demonstrated relatively larger seed sizes, suggesting their suitability for environments with heavy metal contamination.Seed germination is a pivotal stage in plant establishment, particularly under stressful conditions. SBD 377 displayed the highest mean germination percentage of 71.21 ± 1.02, showcasing its resilience to heavy metals stress. IPC 97-72 and ICCV 92944 also exhibited high germination rates, indicating their potential for rapid establishment in contaminated soils.Seed weight influences seedling vigor and early growth. HC 3 demonstrated the highest mean 100-seed weight of 30.28 ± 0.25, suggesting its capacity to provide sufficient energy reserves for germination and early seedling growth under heavy metals stress. BG 267 and ICCV 92944 also showed substantial seed weights, indicating their potential for vigorous growth under adverse conditions.Speed of germination is crucial for seedling emergence and competitive ability. ICCV 92944 displayed the highest mean value of 20.17 ± 2.65, indicating rapid germination and early establishment. HC 3 and Phule G 5 also exhibited fast germination rates, highlighting their suitability for environments with heavy metals contamination.Field emergence reflects the ability of seeds to germinate and establish under field conditions. UD 21 demonstrated the highest mean field emergence of 68.89 ± 0.28, indicating its potential for successful establishment in heavy metal-affected soils. HC 3 and IPC 2004-52 also showed high field emergence rates, suggesting their suitability for cultivation under heavy metals stress.Shoot and root lengths are essential for resource acquisition and plant growth. ICCV 14364 exhibited the longest shoot length (9.62 ± 0.41) and root length (11.16 ± 0.64), indicating its superior growth performance under heavy metals stress. IPC 97-72 and IPC 2004-52 also demonstrated substantial shoot and root lengths, suggesting their potential for deep root penetration and nutrient uptake in contaminated soils.Seedling length and vigour index-I are indicative of overall seedling vigor and growth potential. ICCV 92944 displayed the longest seedling length (12.01 ± 0.56) and highest vigour index-I (1528 ± 67.38), suggesting its superior performance under heavy metals stress. ICCV 88105 and ICCV 14364 also exhibited high seedling lengths and vigour indices, highlighting their potential for robust growth and stress tolerance.

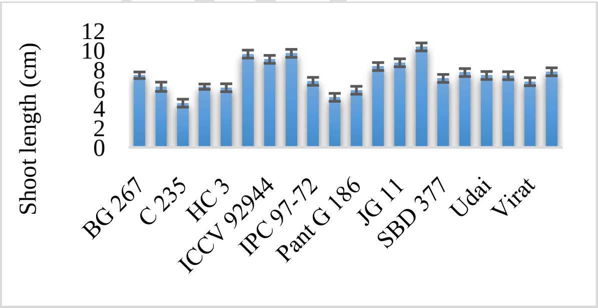
Overall, ICCV 92944 emerges as the superior variety for cultivation under heavy metals stress, exhibiting rapid germination, vigorous seedling growth, and high stress tolerance. However, other genotypes such as HC 3, BG 267, and SBD 377 also demonstrate promising traits and could be considered for breeding programs aimed at developing stress-tolerant chickpea varieties.

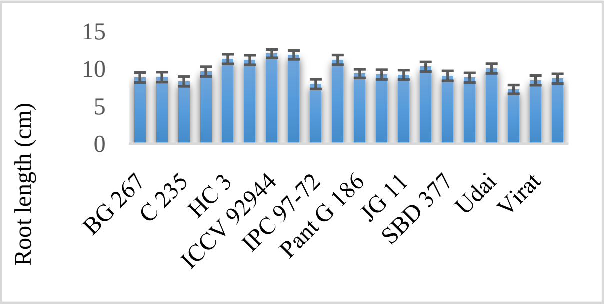












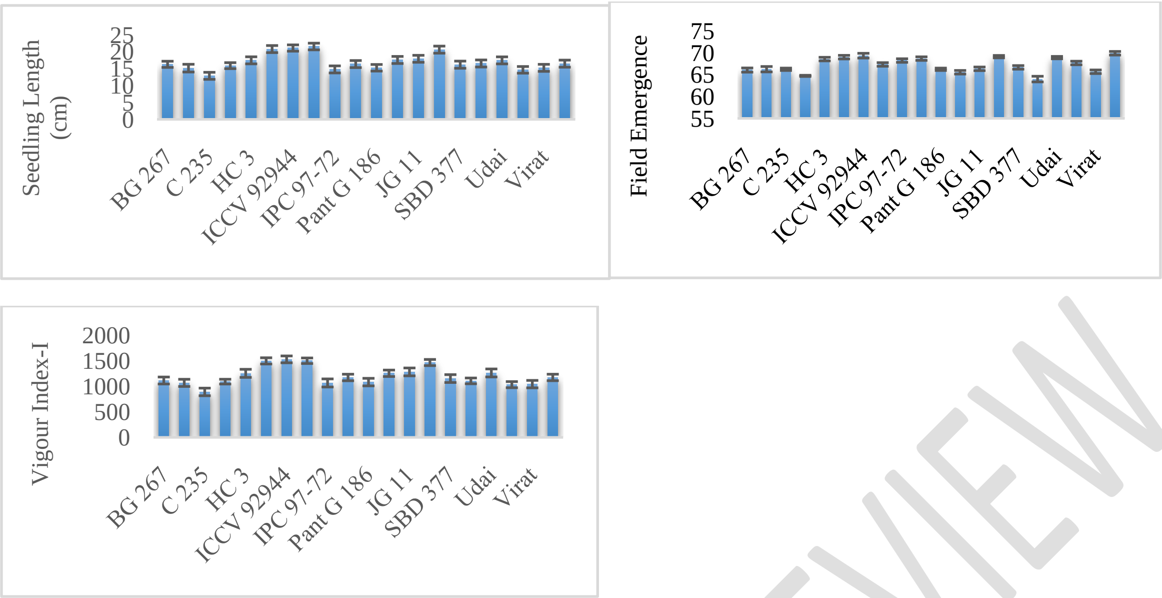


Fig1: Effect of Mn Toxicity on Chickpea Seedling Traits Viz. *Seed Volume*, *100-Seed Weight (g)*, *Seed Germination (%)*, *Speed of Germination*, *Shoot Length (cm)*, *Root Length (cm)*, *Seedling Length (cm)*, *Vigour Index*, and*Field Emergence (%)*

Correlation Analysis of Seedling Traits in Chickpea Germplasm under Heavy Metal Stress

The correlation analysis among various phenotypic traits of chickpea germplasm subjected to heavy metal stress reveals several interesting relationships (Table-1). Seed volume demonstrates a strong positive correlation with 100 seed weight (0.87), indicating that larger seeds tend to have greater weight. Conversely, seed volume exhibits negative correlations with the speed of germination (-0.33) and field emergence (-0.34), suggesting that larger seeds may have slower germination and emergence rates under heavy metal stress. Seed germination shows significant positive correlations with field emergence (0.76), shoot length (0.21), root length (0.38), seedling length (0.35), and vigour index-I (0.48), indicating that higher germination rates correspond to better seedling establishment and growth. Interestingly, 100 seed weight displays a negative correlation with seed germination (-0.38) and speed of germination (-0.50), implying that heavier seeds may germinate and establish more slowly under heavy metal stress conditions. Moreover, shoot length exhibits strong positive correlations with root length (0.36), seedling length (0.85), and vigour index-I (0.83), suggesting that longer shoots are associated with increased root development and overall seedling vigor. Overall, these correlations provide valuable insights into the interrelationships among different phenotypic traits and their responses to heavy metal stress, aiding in the selection of superior chickpea varieties for cultivation in contaminated environments.

Table 1: Correlation Analysis of Phenotypic Traits in Chickpea Germplasm under Heavy Metal Stress

	Seed Volume	Seed Germination	100 seed weight	Speed of germination	Field Emergence	Shoot length	Root length	Seedling length	Vigour Index-I
Seed Volume	1.00	-0.24	0.87	-0.33	-0.34	0.01	0.02	0.02	-0.01
Seed Germination	-0.24	1.00	-0.38	0.34	0.76	0.21	0.38	0.35	0.48
100 seed weight	0.87	-0.38	1.00	-0.50	-0.47	-0.06	-0.18	-0.14	-0.19
Speed of germination	-0.33	0.34	-0.50	1.00	0.43	0.31	0.15	0.28	0.32
Field Emergence	-0.34	0.76	-0.47	0.43	1.00	0.30	0.43	0.43	0.52
Shoot length	0.01	0.21	-0.06	0.31	0.30	1.00	0.36	0.85	0.83
Root length	0.02	0.38	-0.18	0.15	0.43	0.36	1.00	0.80	0.81
Seedling length	0.02	0.35	-0.14	0.28	0.43	0.85	0.80	1.00	0.99
Vigour Index-I	-0.01	0.48	-0.19	0.32	0.52	0.83	0.81	0.99	1.00

Discussion:

The comprehensive analysis of various phenotypic traits in chickpea germplasm under heavy metal stress provides valuable insights into the adaptability and performance of different varieties in adverse environmental conditions. The results reveal significant variations among the studied germplasm for each trait evaluated, reflecting the diverse responses of chickpea genotypes to heavy metal stress.Seed volume, an essential determinant of seedling vigor and nutrient reserves, displayed considerable variation among the germplasm. Varieties such as BG 267 exhibited larger seed volumes, suggesting their potential to provide ample energy reserves for germination and early seedling growth under heavy metal stress. Conversely, other varieties like Subhra showed relatively smaller seed volumes, indicating potential challenges in nutrient acquisition and early growth under similar stress conditions.Seed germination, a critical stage in plant establishment, exhibited notable differences among the germplasm. Varieties such as SBD 377 demonstrated high germination percentages, reflecting their resilience to heavy metal stress and ability to establish rapidly in contaminated soils. In contrast, varieties like C 235 displayed lower germination rates, indicating potential susceptibility to environmental stressors and slower seedling establishment under similar conditions. A similar effect using various heavy metals treatments has been noted for seed germination of several leguminous species such as M. sativa under Cd, Cu, Ni and Pb (Abusriwil*et al*., 2011), A. tortilis, *A. Raddiana* and *P. Juliflora* under Cu and Pb (Abbas *et al*., 2017), A. auriculiformis under Pb (Zerkout*et al*., 2018). Likewise, similar finding has been found for other species belonging to different plant families including, for example: *L. perenne* and *F. rubra* (Poaceae) under Cu, Zn and Co (Taghizadeh and Solgi, 2017), *A. halimus* (Amaranthaceae) under Cd, Zn and Pb (Fatarna*et al*., 2017), *R. sativus* and *B. oleracea* (Brassicaceae) under As (Dutta *et al*., 2014).The 100 seed weight, an indicator of seed size and quality, varied significantly among the studied germplasm. Varieties with heavier seeds, such as HC 3, may possess higher nutrient reserves, contributing to better seedling vigor and early growth under heavy metal stress. However, the negative correlation between 100 seed weight and seed germination suggests potential trade-offs between seed size and germination efficiency, highlighting the need for careful selection in breeding programs to optimize both traits. Speed of germination, an important parameter for seedling emergence and competitive ability, displayed diverse responses among the germplasm. Varieties like ICCV 92944 exhibited rapid germination rates, indicating their potential for early establishment and competitive advantage in heavy metal-contaminated soils. Conversely, varieties with slower germination rates may face challenges in competing with weeds and other crops in similar environments. In this work, the toxicity order of studied metals on FGP, GI, MDG and GV of *M. arborea* seeds was Zn > Cu > Pb. This result is more or less in line with previous researches. Abusriwil*et al*., (2011) reported an order of toxicity for metals on seed germination of *M. sativa* as Cd > Cu > Ni > Pb > Zn. Munzuroglu and Geckil (2002) ranked six heavy metals depending on increasing degree of germination inhibition as Hg > Cd > Cu > Pb > Co > Zn in *Triticum aestivum* grains and as Hg > Cu > Cd > Pb > Zn > Co in *Cucumis sativus* seeds.

Field emergence, reflecting the ability of seeds to germinate and establish under field conditions, varied significantly among the studied germplasm. Varieties such as IPC 97-72 demonstrated high field emergence rates, suggesting their adaptability to field conditions despite heavy metal stress. However, variations in field emergence among different varieties underscore the importance of selecting genotypes with superior establishment traits for cultivation in contaminated environments.Shoot and root lengths, crucial for resource acquisition and plant growth, exhibited considerable variability among the germplasm. Varieties with longer shoots and roots, such as ICCV 14364, may have enhanced nutrient uptake and tolerance to heavy metal stress due to increased surface area for absorption. However, the trade-offs between shoot and root growth highlight the need for a balanced root-shoot ratio to optimize resource utilization and stress tolerance in chickpea varieties.Seedling length and vigour index-I, indicative of overall seedling vigor and growth potential, varied significantly among the studied germplasm. Varieties like Phule G 5 displayed superior seedling lengths and vigour indices, suggesting their potential for robust growth and stress tolerance under heavy metal stress conditions. However, variations in seedling traits among different varieties emphasize the importance of selecting genotypes with optimal growth characteristics for cultivation in contaminated soils.

Overall, the results highlight the complex interactions between different phenotypic traits and their responses to heavy metal stress in chickpea germplasm. The identification of superior varieties with desirable traits such as rapid germination, vigorous seedling growth, and efficient nutrient uptake under stressful conditions is crucial for breeding programs aimed at developing stress-tolerant chickpea cultivars. By leveraging the findings from this study, breeders can prioritize the selection and development of chickpea varieties with enhanced resilience to heavy metal stress, ultimately contributing to sustainable crop production and food security in contaminated environments.

Conclusion:

In conclusion, the evaluation of chickpea germplasm under heavy metal stress revealed ICCV 92944 as the superior variety, showcasing robust traits including high germination rates, 100 Seed weight, Speed of germination, Seedling length and vigour index-I, highlight its potential for resilience in contaminated environments. ICCV 92944's selection offers a promising avenue for breeding programs aimed at developing stress-tolerant chickpea cultivars. By harnessing its genetic advantages, breeders can contribute to sustainable crop production in challenging conditions, ensuring food security and agricultural resilience in the face of environmental stressors.

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Supplementary Table-1: Response of Seedling Traits in Chickpea Germplasm under Heavy Metal Stress

Germplasm	Seed Volume	Seed Germination	100 Seed Weight	Speed of Germination	Field Emergence	Shoot Length	Root Length	Seedling Length	Vigour Index-I
BG 267	33.33 ± 1.04	68.44 ± 2.29	26.79 ± 0.38	20.11 ± 2.21	66.06 ± 0.48	7.44 ± 0.34	8.83 ± 0.66	16.27 ± 0.91	1113 ± 68.55
BG 547	25.33 ± 1.18	70.50 ± 0.69	23.68 ± 0.38	17.58 ± 2.20	66.25 ± 0.62	6.26 ± 0.48	8.88 ± 0.67	15.14 ± 1.14	1067 ± 70.63
C 235	12.33 ± 1.15	69.05 ± 0.52	11.48 ± 0.25	21.43 ± 2.50	66.23 ± 0.28	4.57 ± 0.41	8.30 ± 0.64	12.87 ± 1.05	889 ± 75.00
C821	42.33 ± 0.91	68.73 ± 0.96	30.17 ± 0.25	17.30 ± 2.08	64.73 ± 0.13	6.26 ± 0.28	9.62 ± 0.64	15.88 ± 0.89	1091 ± 46.90
HC 3	37.00 ± 0.25	71.84 ± 0.47	30.28 ± 0.25	18.69 ± 2.09	68.56 ± 0.41	6.16 ± 0.41	11.29 ± 0.64	17.45 ± 1.05	1254 ± 79.39
ICCV 14364	20.00 ± 0.63	72.13 ± 0.71	14.84 ± 0.38	21.04 ± 1.47	69.00 ± 0.41	9.62 ± 0.41	11.16 ± 0.64	20.78 ± 1.05	1498 ± 61.31
ICCV 92944	34.00 ± 0.37	72.48 ± 1.03	22.68 ± 0.25	20.17 ± 2.65	69.34 ± 0.54	9.08 ± 0.41	12.01 ± 0.56	21.09 ± 0.93	1528 ± 67.38
ICCV 88105	17.00 ± 0.39	69.74 ± 0.72	15.87 ± 0.38	21.11 ± 1.80	67.34 ± 0.41	9.70 ± 0.41	11.84 ± 0.59	21.54 ± 0.99	1502 ± 54.49

IPC 97-72	19.00 ± 1.18	72.09 ± 0.36	18.66 ± 0.25	20.06 ± 1.22	68.23 ± 0.41	6.82 ± 0.41	7.95 ± 0.64	14.77 ± 1.05	1065 ± 79.78
IPC 2004-52	19.67 ± 0.75	71.75 ± 0.70	18.76 ± 0.38	19.39 ± 2.36	68.67 ± 0.41	5.17 ± 0.41	11.18 ± 0.64	16.35 ± 1.05	1173 ± 64.09
Pant G 186	15.00 ± 0.76	70.95 ± 0.45	16.23 ± 0.25	17.71 ± 2.09	66.23 ± 0.28	5.90 ± 0.41	9.35 ± 0.58	15.25 ± 0.98	1082 ± 74.16
Phule G 5	25.00 ± 0.25	71.51 ± 0.70	25.72 ± 0.38	18.61 ± 0.91	65.56 ± 0.41	8.34 ± 0.41	9.22 ± 0.64	17.56 ± 1.05	1255 ± 63.04
JG 11	23.67 ± 0.25	71.58 ± 0.54	20.49 ± 0.25	20.39 ± 2.36	66.34 ± 0.41	8.73 ± 0.41	9.18 ± 0.63	17.91 ± 1.03	1282 ± 77.40
JAKI 9218	24.33 ± 0.25	71.13 ± 0.70	22.36 ± 0.25	20.87 ± 2.06	69.11 ± 0.28	10.36 ± 0.41	10.26 ± 0.64	20.62 ± 1.05	1466 ± 60.61
SBD 377	51.33 ± 0.29	71.21 ± 1.02	41.35 ± 0.25	18.69 ± 1.73	66.67 ± 0.41	7.12 ± 0.41	9.05 ± 0.66	16.17 ± 1.06	1151 ± 77.04
Subhra	38.33 ± 0.52	66.97 ± 1.22	34.10 ± 0.25	15.64 ± 2.36	64.00 ± 0.65	7.72 ± 0.41	8.80 ± 0.64	16.52 ± 1.05	1106 ± 56.58
Udai	21.00 ± 0.29	72.34 ± 0.82	18.20 ± 0.25	18.46 ± 2.09	68.89 ± 0.28	7.42 ± 0.41	10.02 ± 0.64	17.44 ± 1.05	1262 ± 78.55
Vaibhav	27.67 ±	70.50 ± 0.72	23.07 ±	21.83 ± 1.47	67.67 ±	7.40 ±	7.24 ±	14.64 ±	1032 ±

	0.39		0.25		0.41	0.41	0.59	0.98	60.20
Virat	27.00 ± 0.29	68.43 ± 1.20	37.82 ± 0.38	17.44 ± 2.36	65.67 ± 0.41	6.78 ± 0.41	8.45 ± 0.64	15.23 ± 1.05	1042 ± 73.30
Vijay	15.33 ± 0.58	71.26 ± 0.70	16.36 ± 0.25	17.42 ± 1.80	69.89 ± 0.41	7.80 ± 0.41	8.67 ± 0.64	16.47 ± 1.05	1173 ± 63.58