

Gravitational and Space Research

Influence of hypergravity on root growth phenotype and physio-biochemical parameters in sorghum (*Sorghum bicolor* L.)

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Abstract

Plants experience the constant pull of gravity on Earth, affecting their growth and development. Recent research has focused on how plants respond to hypergravity, a greater gravitational force compared to 1g. In this study, we examined whether hypergravity could generate new phenotypic traits in sorghum plants. Imbibed sorghum seeds were exposed to varying levels of hypergravity using a centrifuge for short durations, with a 1000g for 1 hour (1000 times Earth's gravity for 1 hour) resulting in enhanced seedling growth and overall plant vigor, both in controlled laboratory settings and greenhouse conditions. Following the screening of several sorghum genotypes, three showed the most promising responses to hypergravity and were further studied. We also investigated the biochemical and hormonal changes triggered by hypergravity. Our findings demonstrated increased enzyme activity in seeds and seedlings, along with elevated chlorophyll levels critical for photosynthesis. Additionally, alterations in the levels of specific plant hormones in the roots, notably 3-indole Acetic Acid and indole-3-butyric acid, appeared to influence root growth. These findings suggest that hypergravity holds the potential for developing novel plant traits with implications for future agricultural advancements.

Keywords

Hypergravity • Sorghum • Root phenotype • Physio-biochemical parameters

Introduction

The force of gravity on Earth (1g) is about 9.807 m/s². This force has been a constant influencing force throughout plant evolution (Brown, 1991). Cells, tissues, and organs are altered by significant deviations from 1g, be it on the lower (microgravity) or higher (hypergravity) side (Merkys & Laurinavicius, 1991; Kiss, 2015). Centrifugation can be used to simulate hypergravity situations where the gravitational force exceeds that of Earth's surface. Plants can successfully regulate their physiological and morphogenic processes in response to different gravitational stimuli because they have developed the capability to perceive gravity, a trait known as gravitropism. Some of these modifications may result in notable phenotypes (Hosamani et al., 2023; Sathasivam et al., 2021). For example, changes in seed germination patterns are one such modification often observed under altered gravitational conditions. In another study, seeds were found to sprout more when subjected to hypergravity (7g for 8 h), followed by the plants being at rest for 16 h at 1g, and were being repeated over four consecutive days (Santos et al., 2012). Other changes, like stronger seedlings, more lignin, and different sugars, have also been noticed in various crops such as radish, cucumber, watercress, beans, carrot, rocket salad, and even the model plant *Arabidopsis* (Kasahara et

al., 1995; Hoson et al., 1996; Soga et al., 1999b; Santos et al., 2012; Russomano et al., 2007; Nakabayashi et al., 2006). Wheat seeds exposed to 500g to 2500g for 10 minutes in an independent investigation showed reversible effects, particularly in post-hypergravity storage circumstances (Dixit et al., 2017, 2022). According to Mshelmbula and Akomolafe (2019), maize seeds subjected to 1000g for 2, 4, and 6 hours showed increased germination percentage, but decreased seedling growth. In contrast, recent research conducted in our laboratory revealed that, in both laboratory and greenhouse conditions, wheat seeds exposed to chronic hypergravity (10g for 12 and 24 hours) significantly increased in total seedling length, root volume, and seedling vigor index compared to control (Swamy et al., 2021; Sathasivam et al., 2022). It is noteworthy that seeds exposed to hypergravity showed higher tolerance to salt stress in cucumbers and carrots. This was observed through increased rates of germination and stronger seedling growth compared to seeds exposed only to salt stress. (Scherer, 2006). Hypergravity has additionally been discovered to strengthen plant cell walls by affecting their properties and components in peas, cress, and azuki beans (Daisuke et al., 2011). Moreover, alongside these alterations in physical traits, hypergravity also affects various physiological and biochemical

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factors. For example, in experiments with chronic hypergravity exposure (at 10g for 25 days and 8 weeks), the photosynthesis rate in *Physcomitrella patens* significantly increased (Takemura et al., 2017). Similarly, rocket salad (*Eruca sativa*) and carrots exposed to hypergravity (17g) showed a notable increase in photosynthesis (Santos et al., 2012). Conversely, wheat and rice seeds exposed to acute hypergravity, and then sown in the normal gravity revealed considerable decrease in the chlorophyll content, photosynthesis rate, transpiration rate, and stomatal conductance (Vidyasagar et al., 2014). Therefore, these investigations suggest that hypergravity can cause variability among phenotypic and physio-biochemical parameters. Most of these studies are restricted to the seedling stage. There is a clear need to examine the possible long-term effects of hypergravity and to evaluate the effect across different genotypes. In the present study we investigated the utility of hypergravity in inducing desired phenotypes and physio-biochemical parameters in seedling stage and vegetative stage of sorghum crop plant. Further, we have validated these phenotypes across different genotypes under greenhouse conditions. We selected sorghum (*Sorghum bicolor* L.) as it is a staple food source for millions of individuals in the semiarid tropics of Africa and Asia.

Materials and methods

Experimental setup for hypergravity treatment on sorghum seeds

The hypergravity treatments were conducted using standard protocols with tabletop centrifuges, as described by Swamy et al. (2021). These centrifuges are capable of creating the desired range of hypergravity—an inertial or centrifugal force in the rotating plane, simulates increased gravity condition. The hypergravity protocol involved placing tubes containing 30 imbibed seeds. Each laboratory experimental condition comprised three replicates such four independent experiments, with a total of 360 seeds (30x3= 90; 90x4 experiments = 360) tested per treatment. The relative centrifugal force (RCF) is referred to as the 'g' force, where 1 RCF equals 1g. For the experiments, freshly harvested seeds of sorghum were obtained from various varieties, including SPV2217, DSV4, M35-1, SVD1418R, Kodmurki local, IS18551, SVD1358R, Lakmapur local, and CSV29R (Figure 6). These seeds were procured from the AICRP sorghum, University of Agricultural Sciences, Dharwad, Karnataka, India.

Experimental protocol for laboratory studies to determine optimal hypergravity intensity in sorghum seedlings for inducing desired phenotypic changes

To determine the optimal hypergravity intensity for inducing desired seedling phenotypes, sorghum seeds were exposed

to various levels of hypergravity such as 20, 40, 80, 100, 250, 500, 1000, 2500, and 5000g for 1 hour. Four independent screening experiments were conducted in triplicate to identify the most effective hypergravity intensity regimen. The desired phenotypes considered for this screening study included germination, seedling vigor, and root and shoot length, as assessed using Davies et al. (2015).

Experimental setup for assessing hypergravity-induced phenotypic changes in sorghum seedlings and roots 55th day in greenhouse condition

To ensure consistency in the effects of the chosen hypergravity intensity and duration regimen at both the seedling and vegetative stages, greenhouse experiments were conducted using various setups. For seedling stage experiment, polythene bags measuring 50 x 20 cm were employed. Individual bags (easier than standard pots for removing roots intact) were filled with a mixture of sterilized 2:6:2 sand, local black soil (as the growth medium), and organic manure. In each bag (one replicate) eight seeds were sown (control and hypergravity treated separately). Regular irrigation with normal water was maintained to keep the soil moisture at field capacity, and perforations on two sides ensured proper drainage. Phenotypic observations were assessed on the 10th day after sowing. This experiment featuring three replications (3 bags x8 = 24 seeds) with four independent experiments [24x4 experiments = 96].

For the assessment of sorghum root phenotype changes specifically after the vegetative growth stage on the 55th day, plastic pipes with a width of 15.24 cm and a height of 91.44 cm were utilized. Individual pipes were filled with the same mixture of 2:6:2 sand, local black soil, and organic manure. In each pipe, five seeds were sown and later thinned out to two [N=48]. Thinning was done randomly to maintain adequate spacing between the plants and also to avoid inter-plant competition for root development and uptake of nutrients. Regular irrigation was maintained to maintain the soil moisture at field capacity. Phenotypic observations were noted on the 55th day after sowing. This experiment was designed with six replicates and four independent experiments for each treatment group and the following parameters were assessed (2x 6=12 and 12x4 experiments = 48 seeds):

- a) *Measurement of shoot and root length*: Shoot and root length, were determined using established methodologies outlined in the International Seed Testing Association (ISTA) Rules of 1999.
- b) *Calculation of seedling vigor index*: The seedling vigor index was computed following the method proposed by Abdul Baki and Anderson (1973) and presented as a numerical index. It is computed by multiplying the percentage of germination by the sum of root length and shoot length.

- c) *Evaluation of seedling dry weight*: The same batch of seedlings used for assessing root and shoot length was placed on butter paper and subjected to drying in a hot-air oven set at 70°C for 24 hours. After cooling in desiccators for 20 minutes, the average dry weight of the seedlings was determined and expressed in milligrams per seedling, based on the method described by Bruns et al. (1985).
- d) *Assessment of root characteristics in sorghum seedlings*: The number of roots per plant and root volume was evaluated solely after the vegetative growth stage, specifically on the 55th day during the greenhouse study. The count of fibrous roots originating from the base of the plant and extending into the soil was conducted following established protocols outlined by Bruns et al. (1985). For root volume determination, the entire root system was immersed in a measuring jar containing a predetermined volume of water. The displaced water was collected, and the volume was quantified and expressed in cubic centimeters (cm³).
- e) *Basic Model #6135*, with methyl cellosolve as a blank (Kittock & Law, 1968).
- c) *Catalase assay in sorghum shoot and root*: The catalase assay involves measuring the absorbance of hydrogen peroxide at 240nm in the UV range. This method depends on the reduction in absorbance caused by the enzyme catalase as it decomposes hydrogen peroxide into water, as described by Hugo and Lester (1984).
- d) *Estimation of superoxide dismutase activity in sorghum shoot and root*: It was assessed by monitoring the reduction in optical density of formazan generated by the reaction between the superoxide radical and nitro-blue tetrazolium dye catalyzed by the enzyme. This method follows the protocol outlined by Dhindsa et al. (1981).
- e) *Phytohormone profiling in sorghum root samples using UPLC-MS/MS*: Phytohormone profiling was outsourced to IIHR, Bangalore, following Pan et al. (2008). Root samples (3 g) from sorghum seedlings (10th day) underwent solid-phase extraction (SPE) before UPLC analysis, and LC-MS/MS (Waters-Acquity, USA) was used for identification and quantification.
- f) *Electrical conductivity*: Counted 50 seeds in each replicate, such three replicates were used for EC assay (N=150 seeds). For each sample to be tested, containers were prepared by adding 250 mL of distilled water. The weighed replicates of seeds were placed into the prepared containers, and each container was swirled to ensure that all seeds were completely immersed. The conductivity of the soak solution was measured at the end of a 24-hour soak period. The conductivity per gram of seed weight for each replicate was calculated by subtracting the background conductivity of the original water from the conductivity reading (dS/m) and dividing the result by the weight (g) of the replicate. The average of the four replicates provides the seed lot test result (ISTA 2015).
- Experimental procedures for assessing physio-biochemical parameters in sorghum**
- a) *Total chlorophyll estimation in sorghum leaves*: The estimation of total chlorophyll was conducted on leaves of both treated and control groups using the method outlined by Barnes et al. (1992). In brief, 0.1 grams of fresh plant leaf material from treated and control samples were placed in test tubes filled with 10 ml of DMSO and left to incubate overnight. The chlorophyll extracted into the DMSO solution was then collected. The total chlorophyll concentration was assessed through a UV-Vis spectrophotometer at wavelengths of 645 nm and 663 nm. The total chlorophyll concentration was quantified using the following formula, which is expressed in milligrams of chlorophyll per gram of tissue.
- $$\text{mg total chl. /g tissue} = 20.2(A_{645}) + 8.02(A_{663}) \times [V/1000 \times W]$$
- [A = Absorbance at a specific wavelength, V = Final volume of chlorophyll extract in DMSO, W = Fresh weight of tissue extracted]
- b) *Assessment of total dehydrogenase enzyme activity in sorghum seeds*: To evaluate dehydrogenase activity, seeds (counted 50 seeds in each replicate, such three replicates were used (N=150 seeds)) were soaked overnight, embryos stained with 0.25% 2,3,5-triphenyl tetrazolium chloride, and then incubated in darkness at 40°C. Following staining, seeds were washed and incubated in methyl cellosolve to extract a red formazan color. The intensity of this color, indicative of dehydrogenase activity, was measured at 470 nm using a UV-Vis spectrophotometer (Eppendorf BioSpectrometer
- Statistical analysis methods employed in the study**
- All data in this research were collected in triplicates to ensure statistical reliability. Data analysis was conducted utilizing SPSS version 20.0, employing one-way ANOVA with critical differences (CD) at 1% and 5% significance levels. Physio-biochemical parameter data were analyzed utilizing an independent variable 'T' test, following the procedure outlined by Sundararaj et al. (1972).

RESULTS

Effect of hypergravity intensities on sorghum seedling growth parameters in laboratory conditions

In the laboratory study, M35-1 sorghum genotype seeds were exposed to various hypergravity intensities ranging from 20g

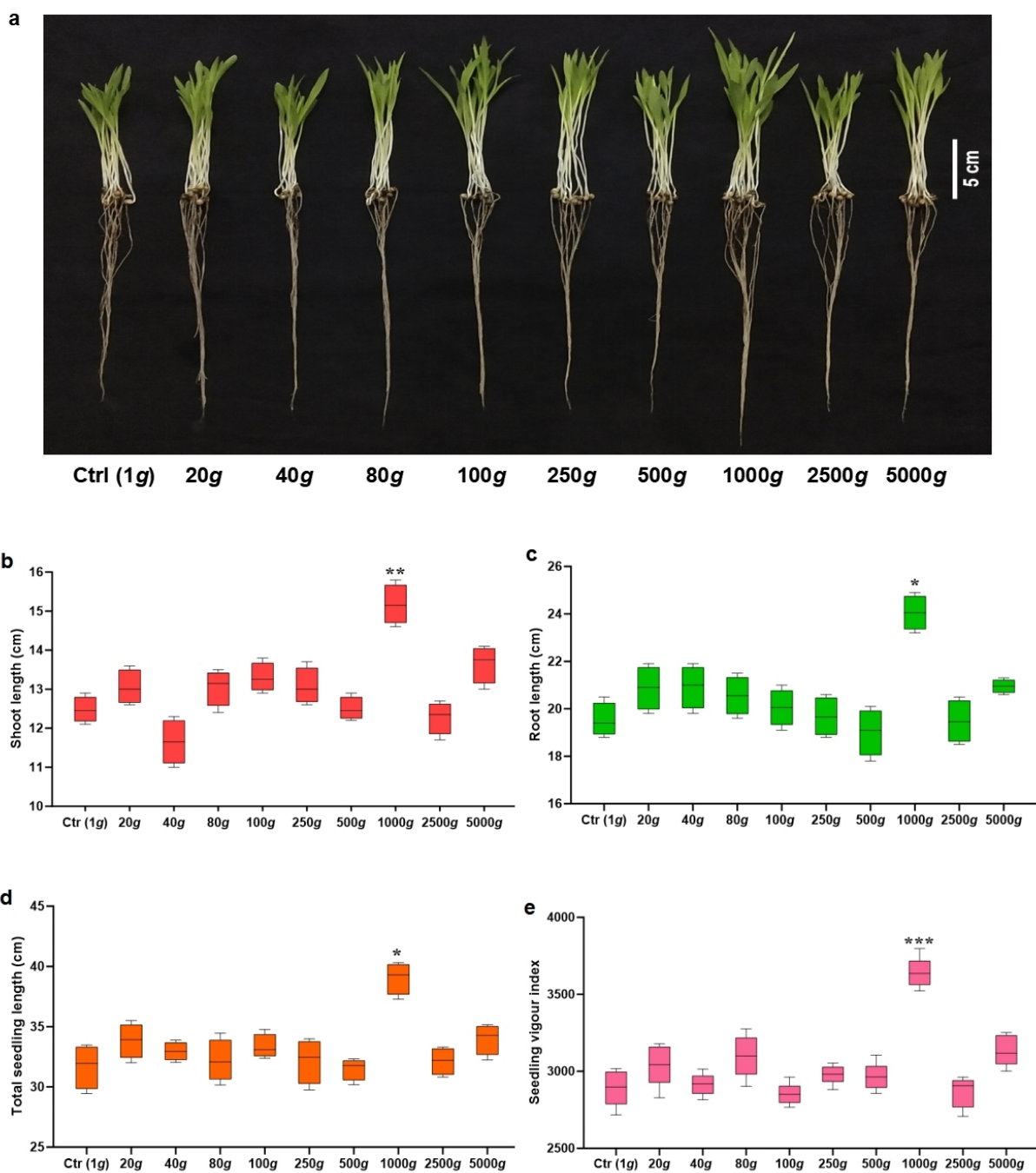


Figure 1. The study evaluated different levels of hypergravity intensities on root, shoot, and seedling vigor index of sorghum seedlings under laboratory conditions using the between-paper method. (a) Visual representation of qualitative root and shoot phenotype responses to 20, 40, 80, 100, 250, 500, 1000, 2500, and 5000-times gravity (g) for a set period of one hour was provided. (b) Variability (shown in red box plot) in the shoot length phenotype among the ten treatments was analyzed. (c) Variability (shown in green box plot) among the ten treatments for root length phenotype was examined. (d) Variability (shown in orange box plot) among the 10 treatments for total seedling length. (e) Variability (illustrated in pink box plot) in the seedling vigor index among the ten treatments was also analyzed. In each test group, 30 seedlings were utilized in triplicates, and such four independent experiments were employed for statistical analysis. The resultant data underwent one-way ANOVA using SPSS – version 20.00. Statistical significance * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

to 5000g for 1 hour. The seeds were subsequently assessed for germination percentage; shoot length, root length, and the seedling vigor index (Figure 1a). The results revealed that only exposure to 1000g for 1 hour resulted in a notable increase in shoot length by 22.38% ($p = 0.011$), root length by 22.75% ($p = 0.023$), and the seedling vigor index by 24.70% ($p = 0.001$) compared to the control (Figure 1b to 1e). None of the other tested intensities induced significant changes in any of the parameters; although, an increasing trend was obtained in some treatment regimes.

Hypergravity treatment (1000g for 1hr) enhances seedling vigor and growth across various sorghum genotypes, in laboratory conditions

After confirming the positive response of the M35-1 sorghum genotype to hypergravity treatment at 1000g for 1hr, we aimed to examine whether this response was exclusive to this specific genotype or if it was consistent across different genotypes. To assess this, we subjected nine different genotypes, including SPV2217, DSV4, M35-1, SVD1418R, Kodmurki local, IS18551, SVD1358R, Lakmapur local, and CSV29R, to the same hypergravity treatment (1000g for 1hr). Among these genotypes, three—SPV2217, M35-1, and IS18551—exhibited a notably similar response to hypergravity, mirroring the findings of our previous study (Figure 2a). Specifically, SPV2217, M35-1, and IS18551 showed significant increases in seedling vigor index by 20.47% ($p = 0.005$), 16.88% ($p = 0.006$), and 11.08% ($p = 0.01$), respectively (Figure 2d). Furthermore, root length was significantly increased by 21.51% ($p = 0.016$), 19.11% ($p = 0.018$), and 18.00% ($p = 0.030$) in SPV2217, M35-1, and IS18551 genotypes, respectively (Figure 2c). Similarly, shoot length exhibited significant increases of 14.92% ($p = 0.046$) and 16.16% ($p = 0.039$) in SPV2217 and M35-1 genotypes, respectively, with no statistically significant change observed in the IS18551 genotype (3.13% ($p = 0.19$)) (Figure 2b). Overall, the total seedling length significantly increased by 19.42% ($p = 0.001$), 17.81% ($p = 0.005$), and 12.31% ($p = 0.003$) in SPV2217, M35-1, and IS18551, respectively (Supplementary table 2).

Greenhouse validation of hypergravity-induced phenotypic changes in selected sorghum genotypes at the 10-day-old seedling stage

After selecting the top three responsive genotypes (M35-1, SPV2217, and IS18551) to 1000g for 1hr, greenhouse validation was conducted. Consistent with previous findings, genotype-specific responses were observed in shoot length, root length, and seedling vigor index (Figure 3a). Notably, M35-1 exhibited a notable increase in shoot length by 14.57% ($p=0.043$), SPV2217 by 10.95% ($p=0.036$), and IS18551 by 1.49% ($p=0.019$) (Figure 3b). Root length increased in M35-1 by 15.06% ($p = 0.010$), and SPV2217 by 13.61% ($p = 0.039$),

with no significant change observed in the IS18551 genotype (2.55%; $p = 0.138$) (Figure 3c). Seedling vigor index showed increases of 16.57% ($p = 0.005$) in M35-1, 9.94% ($p = 0.029$) in SPV2217, and 4.39% ($p = 0.074$) in IS18551 (Figure 3d). Similarly, seedling fresh and dry weight increased in M35-1 by 13.93% ($p=0.039$) and 15.56% ($p=0.028$), SPV2217 by 10.54% ($p=0.035$) and 12.00% ($p=0.034$), and there was no significant change in IS18551 (Figure 3e, 3f), respectively. However, germination remained unaffected (statistically non-significant) in response to hypergravity (Supplementary Table. 3).

Hypergravity-induced phenotypic changes in sorghum genotypes extend beyond the seedling stage in greenhouse conditions

To determine if the phenotypic alterations observed in root and shoot parameters in response to hypergravity could persist beyond the seedling stage, pipe experiments were conducted until the 55th day, marking the end of the vegetative stage in sorghum (Figure 4a). The data revealed that there were no significant changes in germination across the tested genotypes. Interestingly, hypergravity led to notable variations in root length among genotypes. For instance, M35-1 and SPV2217 exhibited increased root length by 19.33% ($p = 0.01$) and 16.62% ($p = 0.015$), respectively, while IS18551 showed a decrease by 18.29% ($p = 0.012$) (Figure 4b). Similarly, root volume significantly increased in M35-1 by 17.42% ($p = 0.026$) and in SPV2217 by 14.67% ($p=0.025$), whereas IS18551 exhibited a decrease by 5.45% ($p=0.011$) as a result of 1000g for 1 hr of treatment (Figure 4c). Additionally, root fresh and dry weight increased in M35-1 by 18.19% ($p=0.01$) and 12.35% ($p=0.003$), and in SPV2217 by 15.08% ($p=0.015$) and 4.91% ($p=0.202$), respectively, whereas IS18551 showed a decrease by 7.09% ($p=0.011$) and 0.05% ($p=0.157$), respectively (Figure 4d, 4e). The number of secondary roots increased in M35-1 by 16.90% ($p = 0.014$) and SPV2217 by 10.87% ($p = 0.017$), whereas IS18551 exhibited a decrease by 3.23% ($p = 0.063$) in response to 1000g for 1 hr (Figure 4f). Furthermore, various shoot parameters were recorded in M35-1, SPV2217, and IS18551 genotypes of sorghum. Notably, significant responses were noticed in shoot parameters in relation to hypergravity treatment. In M35-1, there was an increase in shoot length by 12.36% ($p = 0.025$), shoot fresh weight by 7.65% ($p = 0.032$), and dry weight by 10.44% ($p = 0.02$) in response to 1000g for 1 hr. Similarly, SPV2217 showed an increase in shoot length by 10.85% ($p = 0.016$), with shoot fresh weight and dry weight increasing by 8.64% ($p = 0.023$) and 7.46% ($p = 0.017$), respectively, as a result of hypergravity treatment. In contrast, IS18551, did not exhibit significant changes in the tested phenotypes in response to 1000g for 1 hr (Figure 4g, 4h). Although there was a slight increase in the number of leaves per plant observed across all three genotypes, it was determined to be statistically insignificant.

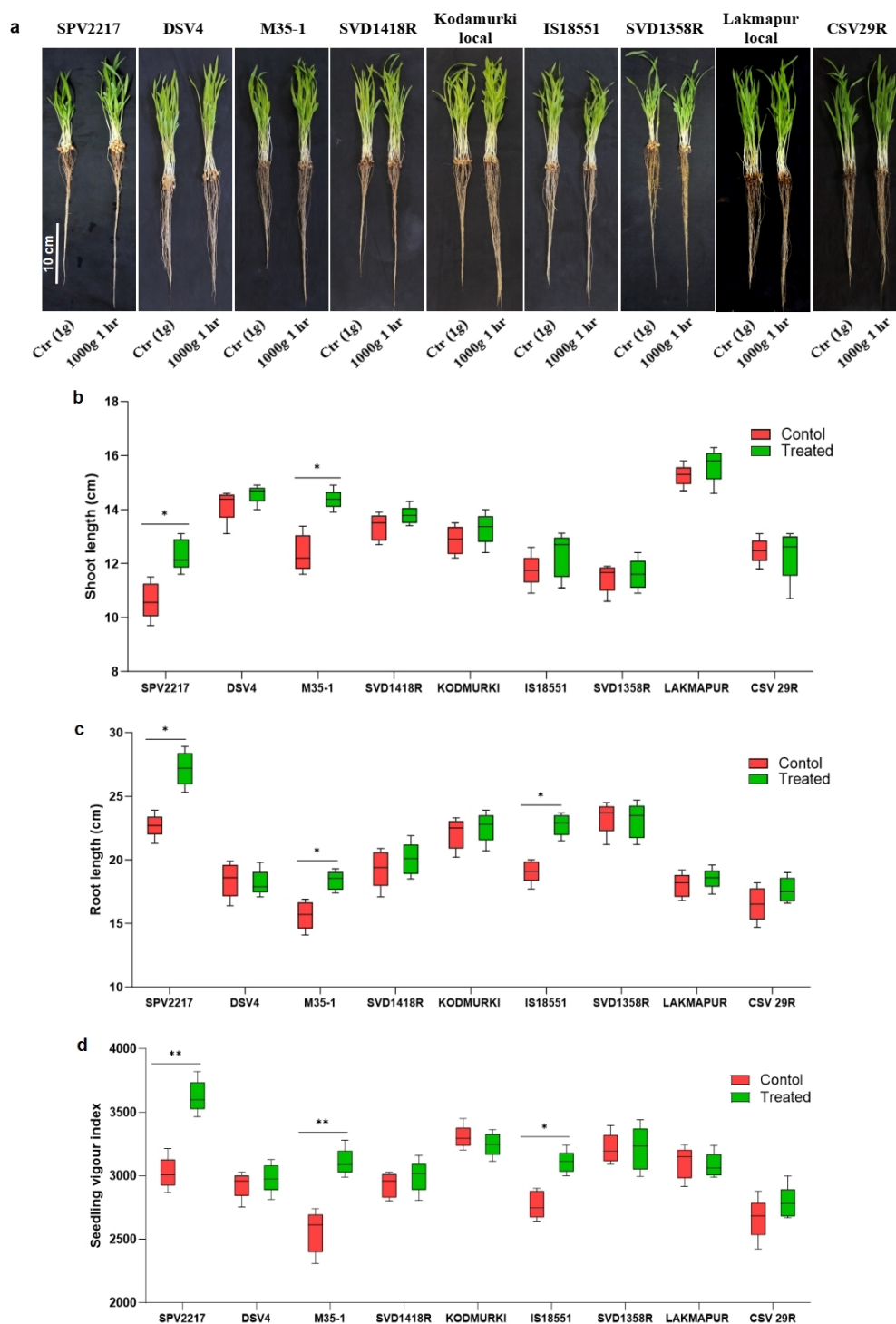


Figure 2. The varied response observed among different sorghum genotypes to hypergravity was examined in laboratory conditions using the between-paper method. (a) A qualitative depiction of hypergravity-induced (1000g, 1 hr) increased root and shoot length phenotypes at the seedling stage were documented on the final count (10th day) of nine different genotypes of sorghum seedlings. Hypergravity-induced alterations in shoot length (b), root length (c), and seedling vigor index (d) of different genotypes of sorghum seedlings were analyzed. The resultant data underwent an independent sample t-test [N=360] using SPSS – version 20.00. Statistical significance * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

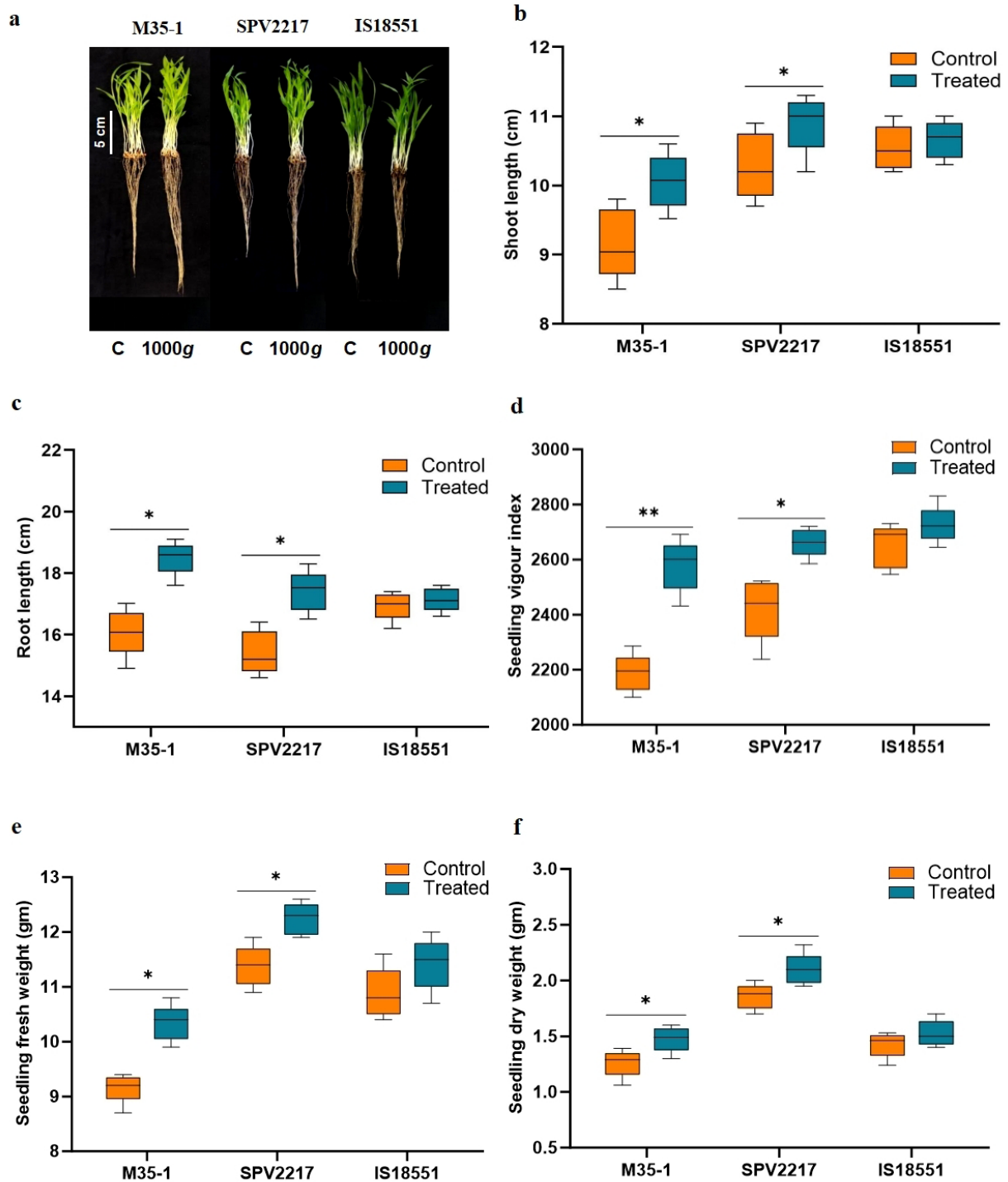


Figure 3. Changes in seedling growth characteristics of three selected sorghum genotypes were examined under greenhous conditions. (a) A hypergravity-induced (1000g, 1 hr) enhanced seedling growth phenotype was recorded at the seedling growth stage (10th day) of sorghum grown in a greenhouse. Hypergravity-induced changes in shoot length (b), root length (c), seedling vigor index (d), seedling fresh weight (e), and seedling dry weight (f) of selected genotypes of sorghum seedlings were analyzed. The resultant data underwent an independent sample t-test [N=96] using SPSS – version 20.00. Statistical significance * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

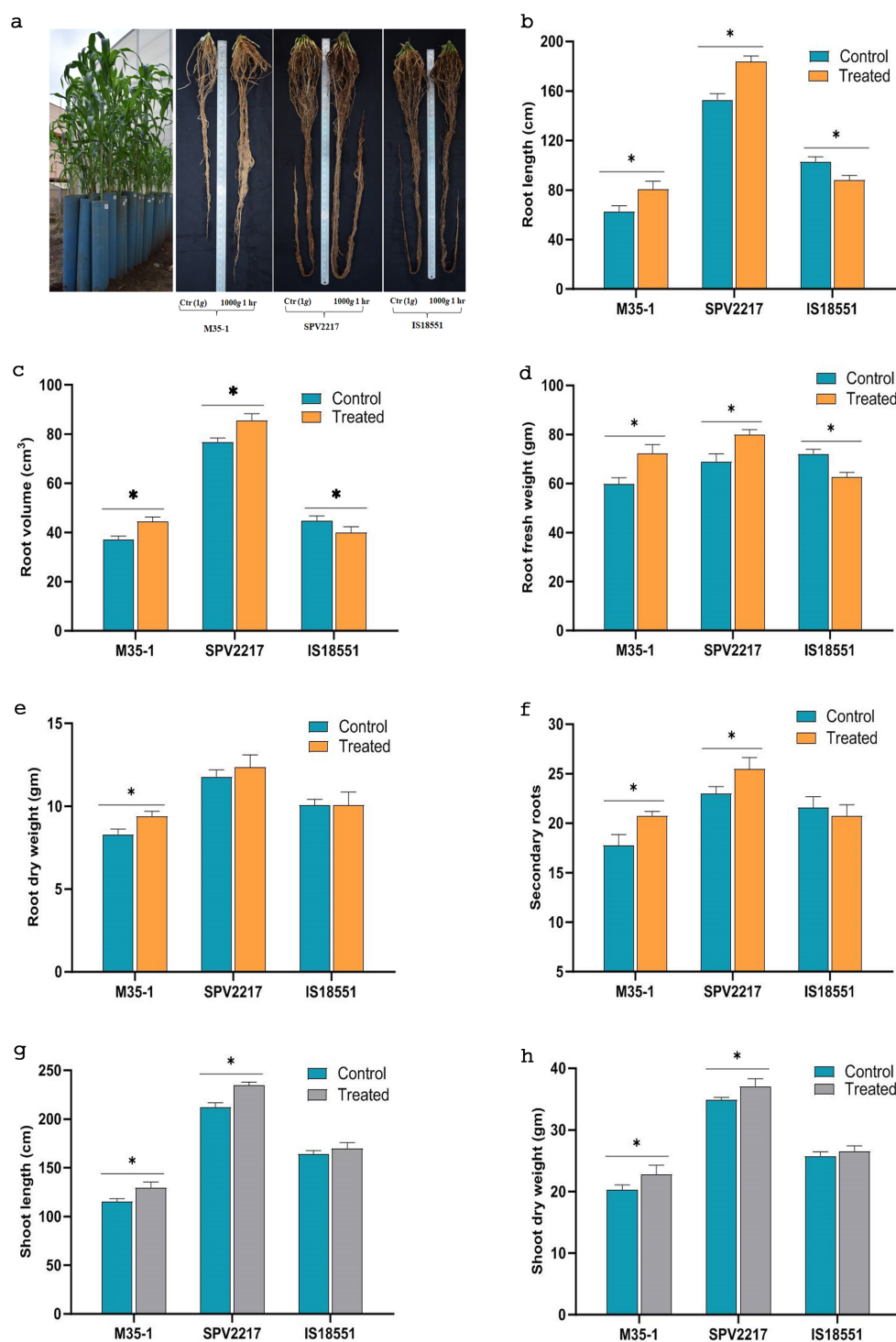


Figure 4. (a) Phenotypic changes were observed at the 55-day-old vegetative growth stage of three selected sorghum genotypes under greenhouse conditions. (b) Hypergravity-induced alterations in root length, (c) root volume, (d) root fresh weight, and (f) secondary roots were analyzed. (g) Hypergravity-induced alterations in shoot length, and (h) shoot dry weight were also assessed. The resultant data underwent an independent sample t-test [N=48] using SPSS – version 20.00. Statistical significance * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

Table 1. Physio-biochemical screening of selected sorghum genotypes. Total chlorophyll content was quantified in leaf tissue; EC and TDH activity were quantified in seeds; catalase and SOD enzyme activities were quantified in seedlings. All biochemical assays were carried out in three replications and four independent experiments were used for an independent variable 'T' test analysis using SPSS – version 20.00 (T-tab. 4.303) * **Statistical significant ($p \leq 0.05$).**

Genotypes/parameters	Ctrl (1g) Mean $\pm$ SEM	1000g 1 hr. Mean $\pm$ SEM	% change over control	p-value	t-stat.
Total chlorophyll content (mg g⁻¹ of tissue)					
M35-1	2.20 $\pm$ 0.19	2.76 $\pm$ 0.04	25.87	0.017*	5.68
SPV2217	2.36 $\pm$ 0.07	2.67 $\pm$ 0.09	13.13	0.038*	4.80
IS18551	2.70 $\pm$ 0.11	3.05 $\pm$ 0.08	12.19	0.023*	8.87
Electrical conductivity (dS/m)					
M35-1	0.29 $\pm$ 0.003	0.28 $\pm$ 0.080	-2.55	0.538 ^{NS}	0.70
SPV2217	0.36 $\pm$ 0.010	0.33 $\pm$ 0.005	-10.23	0.113 ^{NS}	2.34
IS18551	0.23 $\pm$ 0.010	0.21 $\pm$ 0.005	-10.56	0.221 ^{NS}	1.54
Total dehydrogenase (TDH)					
M35-1	0.73 $\pm$ 0.02	0.89 $\pm$ 0.01	21.91	0.010*	8.80
SPV2217	0.74 $\pm$ 0.05	0.86 $\pm$ 0.03	16.87	0.030*	6.19
IS18551	0.81 $\pm$ 0.08	0.92 $\pm$ 0.05	14.71	0.020*	9.20
Catalase (U/ml of enzyme) of genotype M35-1					
Root	5.867 $\pm$ 0.057	7.32 $\pm$ 0.168	24.76	0.008*	8.18
Shoot	5.65 $\pm$ 0.172	6.03 $\pm$ 0.245	6.72	0.282 ^{NS}	1.26
Superoxide dismutase (SOD) (U/ml of enzyme) of genotype M35-1					
Root	1.41 $\pm$ 0.40	1.75 $\pm$ 0.071	24.11	0.023*	4.30
Shoot	2.08 $\pm$ 0.043	2.13 $\pm$ 0.08	2.4	0.589 ^{NS}	0.60

Counted 50 seeds in each replicate, such three replicates were used for EC and TDH assay (N=150 seeds).

For the catalase and SOD activity assays, we used 25 seedlings for preparing homogenate, and such three replicates of homogenate were used for statistical reliability.

Biochemical response of sorghum genotypes M35-1, SPV2217, and IS18551 to hypergravity

The biochemical response of sorghum genotypes (M35-1, SPV2217, and IS18551) to hypergravity (1000g for 1 hr) was evaluated in both seeds and seedlings. In seeds, immediately post hypergravity exposure, total dehydrogenase activity (TDH) increased significantly by 21.91% ($p = 0.01$) in M35-1, 16.87% ($p = 0.03$) in SPV2217, and 14.71% ($p = 0.02$) in IS18551 genotypes in response to hypergravity. However, electrical conductivity showed a decreasing trend in all three genotypes without statistical significance. Additionally, antioxidant enzyme activities were evaluated in the roots and shoots of seedlings of 10-days old to determine physiological stress due to hypergravity exposure. Notably, in the roots, M35-1 exhibited significant increases in catalase by 24.76% ($p = 0.008$) and superoxide dismutase (SOD) by 24.11% ($p = 0.023$) activities as a result of hypergravity treatment, whereas in the shoots, marginal increases were observed with no statistical significance. Additionally, all three genotypes exhibited a notable increase in total chlorophyll

content in response to hypergravity, with M35-1 exhibiting the highest increase by 25.87% ($p = 0.017$), followed by SPV2217 increasing by 13.13% ($p = 0.038$), and IS18551 increasing by 12.19% ($p = 0.023$) (Table 1).

Hypergravity-induced robust phytohormone dynamics and differential responses in M35-1 sorghum genotype

The M35-1 genotype was selected for phytohormone analysis because it showed a significant and consistent response to hypergravity treatment in previous experiments. Quantification of phytohormones in root samples of the M35-1 genotype under hypergravity (1000g for 1 hr) and control conditions using LC-MS/MS revealed distinct responses. Among the 12 phytohormones analyzed, 3-Indole Acetic Acid (IAA), 3-Indole Butyric Acid (IBA), Salicylic Acid (SA), Cis-Jasmonate, and Methyl-Jasmonate showed significant upregulation by 36.59% ($p = 0.019$), 80.18% ($p=0.000$), 57.73% ($p = 0.002$), 30.92% ($p=0.014$), and 87.13% ($p=0.000$), respectively, in response to hypergravity. Conversely, Zeatin trans-isomer, Gibberellic Acid 4 (GA4), Gibberellic Acid 3 (GA3), and

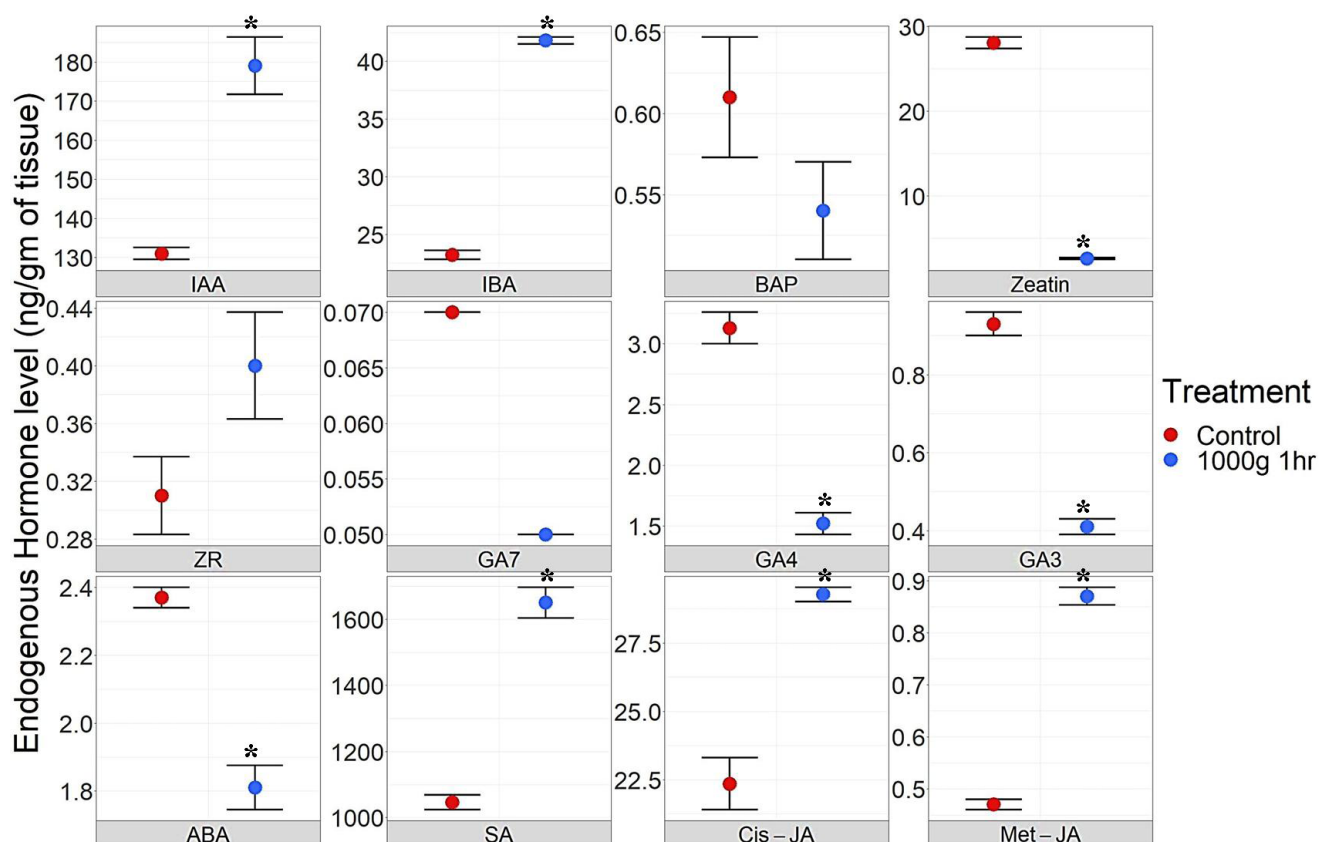


Figure 5. Hypergravity induces dynamic changes in phytohormone levels in the roots of sorghum seedlings, as profiled on the 10th day after exposure. The resultant data underwent an independent sample t-test using SPSS – version 20.00. Statistical significance * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

Abscissic Acid (ABA) exhibited significant downregulation by 90.81% ($p=0.001$), 51.61% ($p=0.001$), 55.75% ($p=0.000$), and 23.40% ($p=0.005$), respectively, in hypergravity-treated samples. The remaining phytohormones, Benzyl aminopurine, Gibberellic Acid 7 (GA7), and Trans zeatin Riboside, showed marginal alterations with no statistical significance compared to the control (decreased by 10.30% ($p=0.271$), 21.51% ($p=0.058$), and increased by 30.66%, $p=0.084$, respectively). The increased auxin levels were positively correlated with improved root growth phenotype under hypergravity conditions. Additionally, elevation in levels of defense hormones such as Salicylic Acid (SA), Cis-Jasmonate, and Methyl-Jasmonate suggest potential physiological stress due to hypergravity (Figure 5).

Discussion

This investigation explores the potential of hypergravity as a novel tool for inducing desirable phenotypes, which could be further utilized to enhance cultivars. While previous studies

have documented altered germination, seedling growth parameters, photosynthesis, physio-biochemical parameters, cell wall thickening, and salt tolerance phenotypes, many of these experiments were limited to seedling stage (Yang Meihong et al., 2005; Russomano et al., 2007). In this study, we evaluate the phenotypes observed in response to hypergravity exposure in seeds and evaluate their potential for improving cultivars beyond the seedling stage under greenhouse conditions, to identify traits that could contribute to enhancing cultivars for terrestrial agriculture.

The preliminary screening studies carried out in M35-1 genotype only indicated that among the various hypergravity intensities tested (ranging from 10g to 5000g for 1hr), the regimen of 1000g for 1hr consistently enhanced shoot length, root length, total seedling length, and seedling vigor in sorghum under both laboratory and greenhouse conditions. Notably, the root length phenotype exhibited consistency across both settings in response to the 1000g for 1 hr treatment. These increases in shoot and root length are particularly significant for biomass production and drought tolerance, respectively. Sorghum, being a fodder crop, stands to benefit from increased biomass



Figure 6. Qualitative image of nine different sorghum genotypes used for this study.

production induced by hypergravity, potentially meeting future demands for fodder. Moreover, the enhancement of root length holds promise for drought avoidance or tolerance, as deeper root systems facilitate improved water and nutrient absorption efficiency (Zaveri & Lobell, 2019). Previous studies have indicated that an improved root phenotype fosters a more beneficial soil microbial community, which in turn enhances plant growth by efficiently modulating nutrient delivery and regulating organ partitioning, such as flower production (Tracy et al., 2020). In our study, the seedling vigor index (SVI) was significantly higher in seeds treated with 1000g for 1 hr compared to untreated seeds. This finding aligns with previous research demonstrating that rocket salad (*Eruca sativa*) and carrot seeds exposed to intermittent hypergravity (17g) exhibited robust seedling vigor phenotypes (Santos et al., 2012). Similarly,

eucalyptus and corymbia seeds exposed to uninterrupted hypergravity treatment at 7g and 5g for 8 and 24 hours, respectively, showed enhanced germination and seedling vigor in the nursery stage (Nunes et al., 2018). Seedling vigor is a critical parameter that defines yield potential, as it facilitates rapid, uniform germination and robust seedling growth across diverse agro-climatic conditions. After confirming the consistent seedling growth phenotypes induced by hypergravity, an investigation was conducted to determine whether these changes were specific to certain genotypes or was observed across a range of genotypes. Nine different genotypes, selected based on their varying susceptibility to drought, were subjected to hypergravity (1000g for 1 hr). Interestingly, three genotypes—M35-1, SPV2217, and IS18551—exhibited a significant enhancement in the root length phenotype in

response to hypergravity. However, the remaining genotypes did not show significant changes, although many of them displayed an increasing trend. This differential response to hypergravity could be attributed to variations in seed size and mass, which interacted with centrifugal force. Subsequently, the M35-1, SPV2217, and IS18551 genotypes of sorghum, which showed the most promising responses, were selected for further investigation of physio-biochemical changes in response to hypergravity (1000g for 1 hr).

The biochemical response of seedlings to hypergravity, specifically at 1000g for one hour, reveals a notable increase in the activities of key antioxidant enzymes, such as catalase and superoxide dismutase (SOD). This observation suggests that the seedlings experience oxidative stress as a consequence of the hypergravity exposure. Oxidative stress increases when there is an imbalance between the production of reactive oxygen species (ROS) and the plant's ability to detoxify these potentially harmful compounds, leading to cellular damage. The heightened activity of catalase and SOD indicates that the seedlings are activating their antioxidant defense mechanisms in response to hypergravity stress. This phenomenon is consistent with similar findings in imbibed wheat caryopses subjected to short-term hypergravity exposure. In this study, wheat seeds exposed to hypergravity intensities ranging from 500g to 2500g for just 10 minutes demonstrated a significant increase in antioxidant enzyme activities (Jagtap & Vidyasagar, 2010). Specifically, catalase and guaiacol peroxidase activities were markedly elevated in these caryopses. Guaiacol peroxidase, a crucial enzyme in the oxidative stress response, helps to break down hydrogen peroxide, a byproduct of various oxidative processes, further indicating an enhanced defensive reaction to hypergravity. Interestingly, this increased activity of antioxidant enzymes persists even after the seedlings or caryopses are returned to normal gravity conditions (1g). For instance, caryopses that were briefly exposed to hypergravity but subsequently grown under standard conditions for five days still exhibited elevated levels of catalase and guaiacol peroxidase. This suggests that the effects of hypergravity induce a lasting adaptive response, potentially preparing the seedlings to better cope with future stressors or fluctuating environmental conditions. The persistence of these increased enzyme activities could be indicative of a prolonged biochemical adaptation or 'memory' effect, whereby the seedlings retain enhanced antioxidant capacity as a result of their previous hypergravity exposure.

Photosynthesis and chlorophyll dynamics in plants hold significant implications for growth, development, and yield. In the current study, total chlorophyll content significantly rose at 1000g for 1hr in the M35-1 genotype compared to the other two genotypes. This observation resonates with a recent study that documented a notable surge in total chlorophyll

content alongside an increase in RUBISCO quantity in wheat in response to hypergravity (10g for 12 hrs) (Swamy et al., 2021). Similarly, prolonged exposure of moss (*Physcomitrella patens*), a model plant, to chronic hypergravity (10g for 8 weeks) resulted in a notable increase in chloroplast size, leading to enhanced photosynthetic rate and biomass (Takemura et al., 2017). Conversely, when subjected to acute hypergravity (100 to 500g for 10 mins and 500 to 3000g for 10 mins), wheat and rice seedlings exhibited a significant reduction in chlorophyll content (Jagtap & Vidyasagar, 2010), indicating a decrease in the photosynthesis rate and associated factors with higher hypergravity intensity (Dixit et al., 2017). These divergent outcomes underscore the significance of employing both acute and chronic hypergravity protocols to elicit favorable physiological traits such as chlorophyll content.

Phytohormones play a pivotal role in orchestrating the growth and morphogenesis of plants. In our study, we delved into the potential correlation between phytohormonal shifts and the altered root phenotype triggered by hypergravity. Employing UPLC/MS-MS, we quantified 12 phytohormones, unveiling significant associations with the observed phenotype alterations. Remarkably, the endogenous levels of 3-indole Acetic Acid (IAA), 3-indole Butyric Acid (IBA), Salicylic Acid (SA), Cis-Jasmonate, and Methyl-Jasmonate exhibited significant upregulation in root samples in response to hypergravity applied at the imbibed seed stage. Notably, the levels of 3-Indole Acetic Acid (IAA) and indole-3-butyric acid (IBA) demonstrated a direct correlation with the regulation of root apical meristem size, root hair elongation, lateral root development, and adventitious root formation (Frick & Strader, 2018). Furthermore, the notable increase in Salicylic Acid (SA), Cis-Jasmonate, and Methyl-Jasmonate levels in response to hypergravity suggests sustained physiological stress induced by hypergravity on plants. In our study, phytohormones including Zeatin trans-isomer, Gibberellic Acid 4 (GA4), Gibberellic Acid 3 (GA3), and Absciscic Acid (ABA) exhibited significant downregulation in samples exposed to hypergravity. Zeatin and its trans isomer (transZ) are bioactive compounds typically found in various plant parts such as the endosperm, root cap, leaf hypocotyl, shoot apex, and endosperm (Gajdosova et al., 2011). Interestingly, previous research has highlighted the antagonistic interaction of Zeatin with root-to-shoot signals (Hansen & Dorffling, 2003). Additionally, root-derived trans-zeatin riboside and absciscic acid have been reported in drought-stressed and re-watered sunflower plants (Hansen & Dorffling, 2003). The correlation between root phenotype and endogenous gibberellins (GAs) levels can be partially explained by the diverse effects exerted by gibberellins, depending on their concentration, as observed in GA-deficient mutants or GA biosynthesis inhibiting studies (Ubeda et al., 2009). ABA is another intriguing phytohormone known to exhibit an inverse relationship with maize root

growth (Mega et al., 2015), thereby correlating the enhanced root length phenotype with dynamic changes in auxin and ABA levels in response to hypergravity (1000g for 1 hr).

Conclusion

This investigation presents evidence of desirable phenotype changes, including increased seedling vigor, root, and shoot length, and positively altered physio-biochemical traits in sorghum genotypes through a novel hypergravity stimulus. Unlike previous studies that are primarily focused on the seedling stage and lacking exploration from the perspective of terrestrial agriculture, our research extends beyond the seedling stage from laboratory conditions to greenhouse settings. We validated the induced phenotypic alterations by hypergravity in various sorghum genotypes. Moving forward, our laboratory aims to further validate these altered phenotypes in field conditions and assess their translational implications on yield enhancement and potential tolerance against abiotic and biotic stresses. This ongoing research holds promise for advancing agricultural practices and improving crop resilience in diverse environmental conditions.

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Authors' contributions

RH, SBH, and DRB conceived the experiment, MAI, SBH and MS experimented, MAI, SBH, RBH, and RH analyzed the results and drafted the manuscript. RH, BB, BKS, and UGR edited the manuscript, and all authors reviewed, read, and approved the manuscript.

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Data availability

Data are available on request from authors.

Disclosure statement

The authors declare that there is no conflict of interest.

Consent to participate

All listed authors have approved the manuscript before submission, including the names and order of authors

Consent for publication

All listed authors have approved the submission of this manuscript to the journal of Gravitational and Space Research.

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Supplementary Table 1. Varied hypergravity intensities evaluated for root, shoot, and seedling vigor index of sorghum seedlings in laboratory conditions. In each test group (Control, 20, 40, 80, 100, 250, 500, 1000, 2500 and 5000g), four independent experimental groups of 30 seedlings in triplicate (30x3 = 90; 90x4 experiments = 360) were used for statistical analysis. Resultant data was subjected to one-way ANOVA using SPSS – version 20.00. * **Statistical significant ($p \leq 0.05$).**

Hyper g treatment	Mean $\pm$ SEM	% change over control	p-value	Cal. F	CD (5 %)	CV (%)
Germination (%)						
Ctr (1g)	90.75 $\pm$ 0.85	-	-	4.20	3.15	2.37
20g 1 hr	90.75 $\pm$ 1.43	0.00	1.000			
40g 1 hr	90.75 $\pm$ 1.25	0.00	1.000			
80g 1 hr	94.75 $\pm$ 0.25	4.41	0.221			
100g 1 hr	87.75 $\pm$ 0.25	-3.31	0.592			
250g 1 hr	89.75 $\pm$ 0.25	-1.10	0.999			
500g 1 hr	92.5 $\pm$ 0.64	1.93	0.970			
1000g 1 hr	94.75 $\pm$ 1.25	4.41	0.221			
2500g 1 hr	91.5 $\pm$ 1.71	0.83	1.000			
5000g 1 hr	93.5 $\pm$ 1.26	3.03	0.699			
Shoot length (cm)						
Ctr (1g)	12.52 $\pm$ 0.04	-	-	41.92	0.43	2.26
20g 1 hr	12.96 $\pm$ 0.08	3.51	0.491			
40g 1 hr	11.77 $\pm$ 0.04	-5.99	0.024			
80g 1 hr	12.99 $\pm$ 0.12	3.77	0.394			
100g 1 hr	13.28 $\pm$ 0.04	6.07	0.211			
250g 1 hr	13.07 $\pm$ 0.08	4.43	0.200			
500g 1 hr	12.47 $\pm$ 0.05	-0.34	1.000			
1000g 1 hr	15.32$\pm$0.17	22.38	0.011			
2500g 1 hr	12.27 $\pm$ 0.07	-2.00	0.959			
5000g 1 hr	13.29 $\pm$ 0.03	6.15	0.239			
Root length (cm)						
Ctr (1g)	19.53 $\pm$ 0.35	-	-	15.67	1.08	3.61
20g 1 hr	21.56 $\pm$ 0.37	6.58	0.230			
40g 1 hr	21.17 $\pm$ 0.39	8.42	0.101			
80g 1 hr	19.71 $\pm$ 0.26	0.92	1.000			
100g 1 hr	20.05 $\pm$ 0.44	2.70	0.992			
250g 1 hr	19.59 $\pm$ 0.64	0.31	1.000			
500g 1 hr	19.03 $\pm$ 0.54	-2.56	0.994			
1000g 1 hr	23.97$\pm$0.31	22.75	0.023			
2500g 1 hr	19.61 $\pm$ 0.34	0.41	1.000			
5000g 1 hr	20.95 $\pm$ 0.27	7.30	0.247			
Seedling vigour index						
Ctr (1g)	2918.0 $\pm$ 22.13	-	-	22.35	138.94	3.15
20g 1 hr	3043.0 $\pm$ 41.23	4.28	0.171			
40g 1 hr	2914.7 $\pm$ 7.57	-0.11	1.000			
80g 1 hr	3099.5 $\pm$ 35.74	6.22	0.063			
100g 1 hr	2850.7 $\pm$ 40.23	-2.30	0.973			
250g 1 hr	2980.5 $\pm$ 20.43	2.14	0.974			
500g 1 hr	2963.7 $\pm$ 40.38	1.57	1.000			
1000g 1 hr	3638.7$\pm$58.45	24.70	0.001			
2500g 1 hr	2906.7 $\pm$ 37.45	-0.39	1.000			
5000g 1 hr	3117.7 $\pm$ 27.98	6.85	0.059			

N= 360 (30 imbibed seeds in each replicate and 3 replicates in each experiment = 90 seeds. 4 independent experiments were carried out, hence 90x4 = 360 seeds).

Supplementary Table 2. Response of different sorghum genotypes to hypergravity under laboratory conditions using between-paper-method. Hypergravity-induced changes in germination, root, length, shoot, and seedling vigor index in different genotypes of sorghum seedlings were recorded on the final count (day 10) of 9 different genotypes of sorghum seedlings. Resultant data was subjected to an independent sample t-test (four independent experimental groups of 30 seedlings in triplicate (30x3 = 90; 90x4 experiments = 360)) using SPSS – version 20.00. * **Statistical significant ($p \leq 0.05$).**

Genotypes	Ctr (1g) Mean $\pm$ SEM	1000g 1 hr Mean $\pm$ SEM	% change over control	p-value	t-stat.
Germination (%)					
SPV2217	91.5 $\pm$ 2.33	92.25 $\pm$ 2.18	0.82	0.835	0.313
DSV4	89.50 $\pm$ 1.45	90.50 $\pm$ 1.20	1.12	0.869	0.177
M35-1	94.50 $\pm$ 1.33	93.75 $\pm$ 1.66	-0.79	0.665	0.469
SVD1418R	89.50 $\pm$ 2.72	91.50 $\pm$ 1.85	2.23	0.709	0.404
KODMURKI LOCAL	93.50 $\pm$ 1.52	90.50 $\pm$ 3.84	-3.21	0.571	0.645
IS18551	91.50 $\pm$ 1.52	90.50 $\pm$ 2.33	-1.09	0.713	0.397
SVD1358R	91.25 $\pm$ 1.52	94.50 $\pm$ 1.33	3.56	0.812	0.981
LAKMAPUR LOCAL	93.75 $\pm$ 1.66	89.50 $\pm$ 2.72	-4.53	0.382	0.548
CSV 29R	91.50 $\pm$ 1.85	93.50 $\pm$ 1.52	2.19	0.617	0.261
Shoot length (cm)					
SPV2217	10.56 $\pm$ 0.70	12.13 $\pm$ 0.83	14.92	0.046	4.51
DSV4	14.38 $\pm$ 0.43	14.69 $\pm$ 0.45	2.16	0.59	0.59
M35-1	12.38 $\pm$ 0.44	14.38 $\pm$ 0.74	16.16	0.039	5.66
SVD1418R	13.65 $\pm$ 0.38	13.78 $\pm$ 0.35	0.92	0.61	0.56
KODMURKI LOCAL	13.10 $\pm$ 0.55	13.37 $\pm$ 0.86	2.02	0.32	1.17
IS18551	11.75 $\pm$ 0.27	12.12 $\pm$ 0.21	3.13	0.19	3.33
SVD1358R	11.67 $\pm$ 0.36	11.80 $\pm$ 0.73	1.16	0.42	0.94
LAKMAPUR LOCAL	15.33 $\pm$ 0.32	15.64 $\pm$ 0.54	2.06	0.31	3.15
CSV 29R	12.48 $\pm$ 0.78	12.62 $\pm$ 0.34	1.08	0.12	2.41
Root length (cm)					
SPV2217	22.70 $\pm$ 0.80	27.58 $\pm$ 0.44	21.51	0.016	4.86
DSV4	18.34 $\pm$ 0.41	18.03 $\pm$ 0.18	-1.68	0.13	2.00
M35-1	15.57 $\pm$ 0.46	18.54 $\pm$ 1.21	19.11	0.018	6.93
SVD1418R	19.15 $\pm$ 1.09	19.19 $\pm$ 1.44	0.21	0.12	2.02
KODMURKI LOCAL	22.23 $\pm$ 0.44	22.50 $\pm$ 0.66	1.20	0.41	3.65
IS18551	18.99 $\pm$ 0.43	22.41 $\pm$ 0.60	18.00	0.030	5.25
SVD1358R	23.33 $\pm$ 0.88	23.48 $\pm$ 0.47	0.68	0.12	3.40
LAKMAPUR LOCAL	18.42 $\pm$ 0.98	18.57 $\pm$ 0.86	0.81	0.97	2.74
CSV 29R	16.51 $\pm$ 0.57	17.16 $\pm$ 1.04	3.89	0.46	3.05
Seedling vigour index (SVI)					
SPV2217	3041.25 $\pm$ 39.6	3663.78 $\pm$ 26.4	20.47	0.005	4.86
DSV4	2929.11 $\pm$ 60.4	2961.63 $\pm$ 51.4	1.11	0.70	1.93
M35-1	2640.16 $\pm$ 36.9	3085.71 $\pm$ 19.4	16.88	0.006	5.995
SVD1418R	2935.45 $\pm$ 13.1	3015.69 $\pm$ 18.6	2.73	0.12	5.19
KODMURKI LOCAL	3303.27 $\pm$ 52.4	3245.50 $\pm$ 53.5	-1.75	0.47	1.42
IS18551	2812.62 $\pm$ 36.3	3124.33 $\pm$ 25.7	11.08	0.01	4.355
SVD1358R	3192.92 $\pm$ 22.3	3333.96 $\pm$ 21.6	4.42	0.09	2.61
LAKMAPUR LOCAL	3163.19 $\pm$ 27.6	3061.30 $\pm$ 20.4	-3.22	0.13	3.90
CSV 29R	2652.75 $\pm$ 15.8	2785.28 $\pm$ 25.7	3.75	0.16	5.28
Total Seedling length (cm)					
SPV2217	33.26 $\pm$ 0.56	39.72 $\pm$ 0.21	20.47	0.005	4.86
DSV4	32.72 $\pm$ 0.16	32.73 $\pm$ 0.09	1.11	0.70	1.93
M35-1	27.94 $\pm$ 0.22	32.92 $\pm$ 0.14	16.88	0.006	5.995
SVD1418R	32.80 $\pm$ 0.14	32.97 $\pm$ 0.30	2.73	0.12	5.19
KODMURKI LOCAL	35.33 $\pm$ 0.22	35.87 $\pm$ 0.25	-1.75	0.47	1.42
IS18551	30.74 $\pm$ 0.39	34.53 $\pm$ 0.12	11.08	0.001	4.355
SVD1358R	34.99 $\pm$ 0.19	35.28 $\pm$ 0.28	4.42	0.09	2.61
LAKMAPUR LOCAL	33.74 $\pm$ 0.25	34.20 $\pm$ 0.17	-3.22	0.13	3.90
CSV 29R	29.00 $\pm$ 0.49	29.78 $\pm$ 0.66	3.75	0.16	5.28

N= 360 (30 imbibed seeds in each replicate and 3 replicates in each experiment = 90 seeds. 4 independent experiments were carried out, hence 90x4 = 360 seeds).

Supplementary Table 3. Changes in germination, root, and shoot growth characteristics of three selected sorghum genotypes under greenhouse conditions. Hypergravity-induced (1000xg, 1 hr) changes in germination, root length (cm), shoot length (cm), seedling vigour index, seedling fresh weight (gm), seedling dry weight (gm) and phenotype recorded at seedling growth stage (10th-day) of sorghum grown in a greenhouse condition. The resultant data was subjected to an independent sample t-test [N=96] using SPSS – version 20.00. * **Statistical significant ($p \leq 0.05$).**

Genotypes	Ctr (1g) Mean $\pm$ SEM	1000g 1 hr Mean $\pm$ SEM	% change over control	p-value	t-stat.
Germination (%)					
M35-1	87.8 $\pm$ 2.51	89.5 $\pm$ 1.15	1.99	0.145	0.361
SPV2217	96.5 $\pm$ 0.00	93.0 $\pm$ 2.33	-3.63	0.116	0.11
IS18551	93.5 $\pm$ 0.66	94.3 $\pm$ 3.00	0.80	0.139	0.108
Root length (cm)					
M35-1	16.1 $\pm$ 0.46	18.5 $\pm$ 1.02	15.06	0.010	6.072
SPV2217	15.4 $\pm$ 0.82	17.5 $\pm$ 0.58	13.61	0.039	4.729
IS18551	16.7 $\pm$ 0.45	17.1 $\pm$ 0.27	2.55	0.138	1.961
Shoot length (cm)					
M35-1	8.8 $\pm$ 0.41	10.0 $\pm$ 1.35	14.57	0.043	4.868
SPV2217	8.5 $\pm$ 1.44	9.4 $\pm$ 0.56	10.95	0.036	4.290
IS18551	10.1 $\pm$ 0.28	10.3 $\pm$ 0.95	1.49	0.079	5.307
Seedling vigour index (SVI)					
M35-1	2195.1 $\pm$ 24.4	2558.8 $\pm$ 20.9	16.57	0.005	5.251
SPV2217	2422.0 $\pm$ 34.1	2662.7 $\pm$ 24.7	9.94	0.029	2.325
IS18551	2635.8 $\pm$ 29.9	2751.4 $\pm$ 33.4	4.39	0.074	1.504
Seedling fresh weight (gm)					
M35-1	9.2 $\pm$ 0.25	10.4 $\pm$ 0.71	13.93	0.039	2.293
SPV2217	11.2 $\pm$ 0.52	12.3 $\pm$ 0.70	10.54	0.035	2.56
IS18551	10.6 $\pm$ 0.18	11.1 $\pm$ 0.23	5.20	0.053	2.527
Seedling dry weight (gm)					
M35-1	1.29 $\pm$ 0.52	1.49 $\pm$ 0.60	15.56	0.028	0.293
SPV2217	1.88 $\pm$ 0.30	2.10 $\pm$ 0.48	12.00	0.034	0.644
IS18551	1.41 $\pm$ 0.35	1.47 $\pm$ 0.47	4.79	0.138	0.284

N=96 (8 seeds in each bag (also referred to as a replicate) and three bags (replicates) total equals 24 seeds. Further, 4 independent experiments were carried out, hence $24 \times 4 = 96$ seeds).

Supplementary Table 4. Changes in germination, root, and shoot growth characteristics of three selected sorghum genotypes under greenhouse conditions. Hypergravity-induced changes in germination (%), root length (cm), root volume (cm³), root fresh weight (gm), root dry weight (gm), number of secondary roots of selected genotypes of sorghum in greenhouse conditions recorded at the beginning of the reproductive phase (55th-day) of sorghum grown in a greenhouse. The resultant data was subjected to an independent sample t-test [N=48] using SPSS – version 20.00. * **Statistical significant ($p \leq 0.05$).**

Genotypes	Ctr (1g) Mean $\pm$ SEM	1000g 1 hr Mean $\pm$ SEM	% change over control	p-value	t-stat.
Germination (%)					
M35-1	94.5 $\pm$ 1.33	95.0 $\pm$ 1.66	0.53	0.566	0.469
SPV2217	93.8 $\pm$ 1.52	94.0 $\pm$ 3.84	0.27	0.179	0.645
IS18551	91.5 $\pm$ 1.52	93.0 $\pm$ 2.33	1.64	0.492	0.397
Root length (cm)					
M35-1	64.9 $\pm$ 0.59	77.5 $\pm$ 0.96	19.33	0.011	13.41
SPV2217	155.0 $\pm$ 1.01	180.7 $\pm$ 0.98	16.62	0.015	13.88
IS18551	103.0 $\pm$ 0.85	87.1 $\pm$ 0.35	-18.25	0.012	24.39
Root volume (cm³)					
M35-1	36.6 $\pm$ 3.96	43.0 $\pm$ 2.34	17.42	0.026	2.927
SPV2217	76.7 $\pm$ 1.10	88.0 $\pm$ 2.52	14.67	0.025	2.964
IS18551	43.6 $\pm$ 2.95	41.2 $\pm$ 1.11	-5.45	0.011	9.571
Root fresh weight (gm)					
M35-1	65.4 $\pm$ 0.86	71.3 $\pm$ 0.55	18.19	0.01	11.32
SPV2217	69.0 $\pm$ 0.58	79.4 $\pm$ 0.98	15.08	0.015	12.43
IS18551	72.2 $\pm$ 0.54	61.2 $\pm$ 0.32	-7.09	0.011	9.85
Root dry weight (gm)					
M35-1	8.3 $\pm$ 1.35	9.3 $\pm$ 3.28	12.35	0.003	4.32
SPV2217	11.7 $\pm$ 2.71	12.3 $\pm$ 2.87	4.91	0.202	5.17
IS18551	10.0 $\pm$ 1.49	10.1 $\pm$ 2.16	0.05	0.157	4.78
Number of secondary roots					
M35-1	15.8 $\pm$ 1.58	20.8 $\pm$ 4.71	16.90	0.014	4.431
SPV2217	23.8 $\pm$ 1.83	25.5 $\pm$ 3.93	10.87	0.017	3.274
IS18551	23.3 $\pm$ 1.65	22.5 $\pm$ 1.47	-3.23	0.063	2.967
Shoot length (cm)					
M35-1	115.3 $\pm$ 7.2	129.5 $\pm$ 7.69	12.36	0.025	4.333
SPV2217	212.0 $\pm$ 13.5	235.0 $\pm$ 12.9	10.85	0.016	7.534
IS18551	164.0 $\pm$ 10.5	169.5 $\pm$ 17.2	3.35	0.059	9.532
Number of leaves per plant					
M35-1	09 $\pm$ 0.40	9.3 $\pm$ 0.51	2.78	0.134	1.732
SPV2217	11 $\pm$ 0.40	11.3 $\pm$ 0.54	2.27	0.235	1.543
IS18551	13 $\pm$ 0.86	13.5 $\pm$ 0.12	3.85	0.323	1.786
Shoot fresh weight (gm)					
M35-1	151.6 $\pm$ 1.9	163.2 $\pm$ 1.3	7.65	0.032	4.928
SPV2217	217.6 $\pm$ 1.7	236.4 $\pm$ 2.5	8.64	0.023	1.206
IS18551	188.6 $\pm$ 1.2	199.8 $\pm$ 3.4	5.53	0.129	2.863
Shoot dry weight (gm)					
M35-1	20.6 $\pm$ 0.08	22.8 $\pm$ 0.25	10.44	0.02	5.23
SPV2217	34.9 $\pm$ 0.27	37.5 $\pm$ 0.52	7.46	0.017	7.121
IS18551	25.8 $\pm$ 0.37	26.5 $\pm$ 0.51	2.81	0.204	4.439

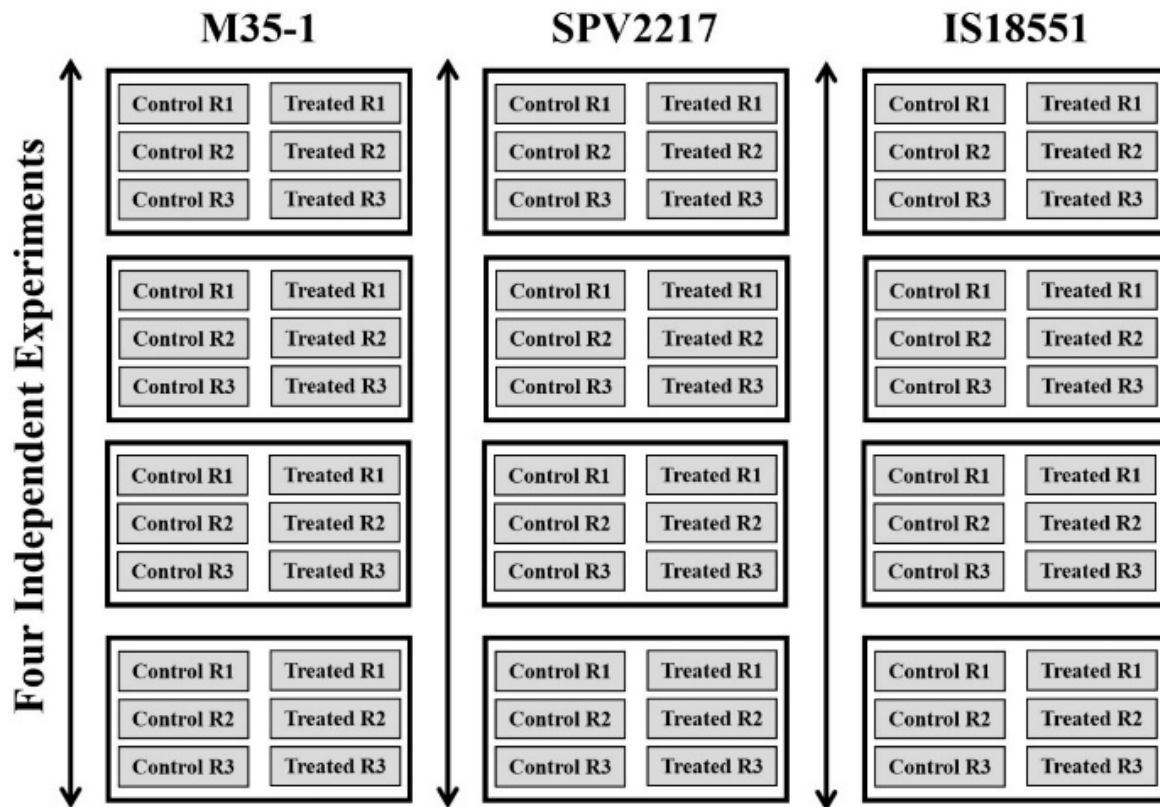
N= 48 (2 seeds in each pipe (replicate) and six pipes (6 replicates) were used, comprising 12 seeds in each experiment. Further, 4 experiments were carried out, so 12x4= 48 seeds.

Supplementary Table 5. Hypergravity elicits robust phytohormone dynamics in the root, profiled on the final count (day 10) of sorghum seedlings. Resultant data was subjected to an independent sample t-test using SPSS – version 20.00. * **Statistical significant ($p \leq 0.05$).**

Hormones	Phytohormones (ng/gm of tissue)				
	Ctr (1g) Mean $\pm$ SEM	1000g 1 hr. Mean $\pm$ SEM	% change over control	p-value	t-stat.
Indole Acetic Acid (IAA)	131 $\pm$ 1.54	179 $\pm$ 7.33	36.59	0.019	6.396
Indole Butyric Acid (IBA)	23.20 $\pm$ 0.39	41.81 $\pm$ 0.29	80.18	0.000	38.18
Benzyl aminopurine (BAP)	0.61 $\pm$ 0.03	0.54 $\pm$ 0.03	-10.30	0.271	1.278
Zeatin transe isomer	28.05 $\pm$ 0.68	2.58 $\pm$ 0.09	-90.81	0.001	37.12
Trans zeatin Riboside (ZR)	0.31 $\pm$ 0.02	0.40 $\pm$ 0.03	30.66	0.084	2.603
Gibberellic Acid 7 (GA7)	0.07 $\pm$ 0.00	0.05 $\pm$ 0.00	-21.51	0.058	2.858
Gibberellic Acid 4 (GA4)	3.13 $\pm$ 0.13	1.52 $\pm$ 0.09	-51.61	0.001	10.11
Gibberellic Acid 3 (GA3)	0.93 $\pm$ 0.03	0.41 $\pm$ 0.02	-55.75	0.000	15.30
Absciscic Acid (ABA)	2.37 $\pm$ 0.03	1.81 $\pm$ 0.06	-23.40	0.005	7.506
Salicylic Acid (SA)	1046 $\pm$ 22.06	1650 $\pm$ 46.16	57.73	0.002	11.80
Cis-Jasmonate (cis-JA)	22.35 $\pm$ 0.95	29.27 $\pm$ 0.26	30.92	0.014	6.992
Methyl-Jasmonate	0.47 $\pm$ 0.01	0.87 $\pm$ 0.01	87.13	0.000	17.71

Supplementary Table 6. Seed Diameter and 100 Seed Weight Across Different Sorghum Genotypes.

Sl. No.	Sorghum Genotypes	Seed Diameter (mm)	100 Seed Weight (g)
1	SVD1418R	1.6	3.85
2	IS18551	1.3	2.67
3	SVD1358R	1.4	4.34
4	DSV4	1.5	3.55
5	LAKMAPUR LOCAL	1.5	3.10
6	SPV2217	1.6	4.15
7	M35-1	1.6	4.16
8	CSV 29R	1.5	3.52
9	KODMURKI LOCAL	1.4	3.35



Supplementary Figure 1. This experiment was grouped as below. Statistical superiority was identified using an independent 't' test comparing control with hypergravity treatment.