

Phytic Acid is Indispensible in Plant's Perspective: Evidence with Finger Millet (*Eleusine coracana* L.)

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ABSTRACT

Grain yield under rainfed conditions is predominantly determined by the extent of seed germination, plant stand and seedling vigour, which in turn depends on the seed reserves. One important seed reserve is grain phosphorus (P) stored in the form of phytic acid (PA). From the plant's perspective, high grain PA-P promote seed germination and seedling establishment. Contrastingly, high PA (HPA) limits the bioavailability of micronutrients like iron, zinc and other cations in human and monogastric animals. Therefore, the present study was conducted to verify whether the seedling parameters of finger millet are dependent on grain PA content. If so, identifying low PA (LPA) genotypes with better seedling vigour index (SVI) could be useful for both human and plant perspectives. Grain PA was ranged from 0.84 mg/g (GE-3767) to 14.1 mg/g (GPU-28). The HPA genotypes maintained higher seed germination, seedling length, seedling weight, SVI, total dehydrogenase activity (DHA) and alpha-amylase activity than the LPA genotypes. Furthermore, the PA had a significant positive relationship with SVI ($r=0.715^{**}$) and grain yield ($r=0.431^{*}$) and the SVI had a positive significant influence on grain yield ($r=0.628^{**}$). The identified low PA (<1.8 mg/g) genotypes, GE-3767, GE-2154 and GE-3094, had only 11.8 per cent of PA with a SVI of 46.3 per cent compared to the popular variety, GPU-28. These lines can be used as donors for improving the bioavailability of micronutrients.

Keywords : Phytic acid, Seedling vigour, Dehydrogenase, Alpha amylase, Grain yield

Small millets have the potential to improve food security, nutrition and livelihoods, especially in dry and marginal lands (Muthamilarasan and Prasad, 2021). Finger millet is the most popular, due to higher productivity compared to other small millets (<http://www.indiaagrstat.com>). Finger millet is a rainfed crop in more than 90 per cent of the cultivated area in India (Davis *et al.*, 2019). It has better drought resilience than other major cereals and suitable for climate change scenarios (Chaturvedi *et al.*, 2022). In addition, finger millet has superior nutritional quality (Chandra *et al.*, 2016; Hiremath *et al.*, 2018) with low glycemic index (Chaturvedi *et al.*, 2022). However, climate change-induced frequent droughts accompanied by high temperatures led to stagnation

in yield improvement of finger millet over the past two decades (Adugna *et al.*, 2011; Saxena *et al.*, 2018; Megha *et al.*, 2023). The drought conditions reduce the grain yield to the extent of 37.6 per cent depending on the stage of the crop, duration and intensity of the drought (Krishna *et al.*, 2021 and Nanja Reddy & Priya Reddy, 2023) and at high temperature above 32°C (Yogeesh *et al.*, 2016; Opole *et al.*, 2018 and Ramya & Nanja Reddy, 2018). Under rainfed conditions, grain yield is determined, primarily by the extent of seed germination, plant stand and seedling vigour (Nanja Reddy *et al.*, 2022). The higher seedling vigour maintains an adequate plant population, suppresses weed growth and reduces soil evaporation, thus favours water availability, promoting growth and

productivity under rainfed conditions (Fahad *et al.*, 2017; Nanja Reddy *et al.*, 2021).

The seedling vigour mainly depends both on the seed reserves and external climatic conditions. Among which seed reserves are more important for early seedling vigour. Finger millet is generally grown on poor soils with low organic carbon, macro and micronutrients. Under such conditions, plants have evolved strategies to accumulate phosphorus (P) in the form of phytic acid (PA) in the seeds and a low P limits seed germination, seedling vigour and crop establishment (Yugandhar *et al.*, 2022). But, in soils rich or amended with P, the seed P content does not have an advantage on seed germination and seedling vigour but has a significant role under P deficit soils (Rose and Raymond, 2020). In India, 53 per cent of soils are classified as low in available P, 30 per cent as moderate, and 12 per cent as high (Sanyal *et al.*, 2015). Therefore, external application of P is inevitable (although the majority of farmers apply only the nitrogen) and such P will be fixed in the soil (become non-available) or leach down to cause eutrophication of water bodies (Boesch *et al.*, 2001). Contrastingly, in humans, high grain P is an anti-nutritional factor that limits the bioavailability of cations like iron, zinc and other elements due to the lack of phytase enzyme (Hejadi *et al.*, 2016; Reddy *et al.*, 2022). Hence, it would be appropriate to address the role of seed P level on seedling vigour, which helps to reduce P fertilization, and it would be worthwhile to identify the critical limit of P without affecting the seedling vigour markedly. Hence, in the present study, genotypes differing in grain yield under rainfed situations were evaluated for their seed PA and its influence on seedling vigour and associated enzyme activities to identify LPA genotypes.

MATERIAL AND METHODS

The experiment was conducted at the Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bengaluru, during 2017. Seeds of 24 genotypes with a wide range of yielding abilities under rainfed conditions were evaluated for PA, dehydrogenase activity, alpha-amylase and seedling

vigour parameters in a completely randomized design in three replications.

Seed Germination and Seed Quality Parameters

Freshly harvested seeds were treated initially with a 1.5 per cent sodium hypochloride solution for 15 minutes for surface sterilization to remove fungus and bacteria and to remove partially filled seeds. The good seeds were washed thrice in distilled water and used for germination. For the seed germination test, a polythene sheet was spread, over which a germination paper was spread and thoroughly wetted using distilled water. On such paper, 100 seeds were placed for germination. Then rolled and placed in a growth chamber for 7 days, at a temperature of $25 \pm 1^\circ\text{C}$ (day and night) and 90 per cent relative humidity, the method is called between paper towel method. The observations were made on the 8th day after sowing. Seeds that produced a radicle length of at least 5 mm were called normal seedlings and were counted as germinated. From the normal seedlings, 10 seedlings were selected randomly for measuring root length and shoot length. After measuring the length the seedlings were kept for oven drying at 70°C for 17 hrs and seedling dry weight was recorded. Considering the germination percentage, seedling length and seedling dry weight, the SVI-I (seed germination percentage \times seedling length in cm) and SVI-II (seed germination percentage \times seedling dry weight in mg) were calculated as suggested by Abdul-Baki and Anderson (1972).

Phytic Acid (PA)

PA was quantified colourimetrically by the modified Wade reagent method (Gao *et al.*, 2007). Dried seed samples were ground in a mortar with a pestle using liquid nitrogen. Samples of 0.50 g of ground powder were transferred to 30 mL Falcon tubes and were thoroughly mixed with 10 ml of 2.4 per cent HCl. Sample tubes were then kept on shaker at 220 rpm for 16 hours at room temperature. Then the tubes were centrifuged at 1000 g (2500 rpm) at 10°C for 20 minutes and the supernatant was transferred to a fresh tube. Collected supernatant (One mL) is diluted 25 times in a 50 mL Falcon tube by adding 24 mL of

distilled-deionized water). A 3 mL of this diluted sample was combined with 1 mL of modified Wade reagent (0.03% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ + 0.3% sulfosalicylic acid), thoroughly mixed on a vortex for 5-10 seconds and centrifuged at 1000 g at 10°C for 10 min. The absorbance of the supernatant was measured at 519 nm. For standard curve preparation, a series of calibration standards containing 3 µg to 24 µg PA standards in 3 mL water and 1 mL of modified Wade reagent was added.

Dehydrogenase Activity (O.D)

During the germination process, there will be a higher respiration rate, which can be measured by the reduction of TTC (colorless 2,3,5- tetrazolium-tri-chloride) to formazan (red coloured 1,3,5- tetrazolium-tri-chloride), called as TZ test, this reaction is mediated by dehydrogenase enzyme. The total dehydrogenase activity (DHA) test was performed as per Kittock and Law (1968).

One gram of seed was ground to fine powder to pass through a 20-mesh screen in three biological replications. Two hundred mg flour (for quick penetration of TTC into flour compared to the seed) was soaked in 5 mL of 0.5 per cent tetrazolium solution at 38°C for 3 hours. Then it was centrifuged at 10,000 rpm for 3 minutes and the supernatant was poured off and the residue was collected. To the residue, 10 mL of acetone (absolute) was added and left for 16 hours, centrifuged at 10,000 rpm for 3 minutes and the supernatant was used to read absorbance at 480 nm. The intensity of colour will be proportional to the DHA.

Alpha-amylase Activity in Seeds

Amylase activity was determined by the DNS reagent. For this, 2 g of 3,5-dinitro salicylic acid (DNS) was suspended in 40 mL of 2N NaOH. To this, 100 mL of 60 per cent sodium potassium tartrate solution was added. The final volume was made up to 200 mL using distilled water. The reagent was filtered and stored in a dark bottle.

Standard Glucose Solution : 40 mg of glucose was dissolved in 100 mL of distilled water in a standard volumetric flask to get a standard glucose solution of 400 µg/mL.

One Per cent Starch Solution : 1g of starch was made into a paste with little water. It was then added to boiling water with continuous stirring. The solution was cooled and made up to 100 mL with distilled water.

Enzyme assay (0.1M phosphate buffer of pH 7.5): (a) Stock A: 2.685 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ in 100 ml of distilled water to get 0.1M solution. (b) Stock B: 1.39 g of $\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ in 100 ml of distilled water to get 0.1M solution. Stock A and B were mixed to get a pH of 7.5

Sample Preparation : Seeds of finger millet were soaked in water for zero and 15 hours and the amylase activity was determined. Before the seeds were soaked in water, a known amount of seeds were taken and at the end of the imbibition period of 15 hours, 0.1 g of seeds were taken and homogenized with 0.9 mL of phosphate buffer using pestle and mortar in ice bath.

Measurement of Alpha-amylase Activity : A 1.5 mL of 0.1M phosphate buffer of pH 7.5 was added to 1 mL of starch (1%) and the mixture was incubated at room temperature for 5 minutes. In all the test tubes, the reaction was started by adding 0.5 mL of the enzyme extract. To the other test tube, 0.5 mL of killed enzyme was added (enzyme was killed by heat) and this served as blank. The reaction was allowed to continue for 20 minutes in all the test tubes. The reaction was stopped by the addition of 2 mL DNS reagent to each test tube. The test tubes were placed in a boiling water bath for 10 minutes and cooled. The optical density of the orange-yellow coloured complex so obtained was measured at 540 nm and the values were recorded. The OD values were plotted against the standard graph of glucose to determine the amount of glucose which is a direct reflection of alpha amylase activity.

Statistical Analysis

The ANOVA was performed in CRD using the software, OPSTAT (Sheoran *et al.*, 1998). The correlations and the figures were done with the Microsoft XL.

RESULTS AND DISCUSSION

Phosphorus (P) is the major element that is essential for plant growth and development and most soils in India are deficient in P (Sanyal *et al.*, 2015). Therefore, external application is obligatory and the increased P application up to 40 kg/ha was found to increase the grain yield of finger millet (Krishna *et al.*, 2019 and Dharmendra & Umesha, 2022). However, finger millet being a rainfed crop, farmers rarely apply fertilizer; hence, seed P is important for seed germination, seedling vigour, plant growth and yield under rainfed situations (Oo *et al.*, 2023).

Phytic Acid

Globally micronutrient malnutrition especially, the iron and zinc are the most serious health constraint elements for humans (Gupta *et al.*, 2015). This is mainly due to the presence of anti-nutrients (the phytic acid, PA that accounts for 65-85% of total phosphorus

in the food grains; Kumar *et al.*, 2021). The PA is myo-inositol 1,2,3,4,5,6-hexakisphosphate containing six negative charges, binds six cations like calcium, iron, zinc, manganese, magnesium and potassium and reduces the absorption of cations by human and monogastric animals like poultry and pigs due to lack of phytase enzyme for breakdown of PA (Gupta *et al.*, 2015 and Kumar *et al.*, 2021). In contrast, the plants do accumulate PA in grains for their better seed germination and seedling vigour in subsequent generation (Yugandhar *et al.*, 2022). In the present study, the seed phytic acid (PA) was significantly higher (11.12 mg/g) in high grain yielding genotypes compared to low yielding genotypes (7.36 mg/g) and the highest PA was found in popular variety GPU-28 (14.1 mg/g). The least PA was observed in the low grain yielding accessions, GE-2154 (1.65 mg/g), GE-3094 (1.80 mg/g) and GE-3767 (0.84 mg/g; Table 1). The present results corroborate the similar range from 5.94 to 8.93 mg/g (Hiremath and Geetha,

TABLE 1
Phytic acid, seedling vigour and associated parameters in studied finger millet genotypes

Genotype	Grain yield (g m ⁻²)	PA	G (%)	RL (cm)	SL (cm)	TSL (cm)	Seed-ling DW	SVI-I	SVI-II	Mean SVI	DHA
GE-4568	584.8	9.59	98.3	12.8	6.6	19.4	18.1	1909	1784	1846	0.717
L-5	620.0	8.69	95.7	8.6	6.8	15.6	17.8	1480	1703	1592	0.364
GPU-28	411.7	14.10	99.3	12.4	6.1	18.6	20.2	1836	2010	1923	0.638
GE-5117	563.5	7.84	100.0	11.3	6.6	17.9	16.6	1790	1657	1723	0.519
GE-1077	526.1	12.67	96.7	12.8	7.2	20.0	17.6	1930	1697	1813	0.718
GE-436	535.2	10.37	97.0	10.9	6.6	17.6	15.5	1701	1503	1602	0.638
GE-199	557.2	12.61	100.0	12.8	7.4	20.2	22.4	2023	2243	2133	0.891
GE-1026	528.5	11.33	95.3	6.2	7.6	13.8	12.7	1320	1206	1263	0.168
GE-4683	494.4	11.74	92.7	10.1	7.5	17.6	18.8	1631	1741	1686	0.455
GE-4004	542.2	10.30	96.7	10.4	7.5	17.9	19.9	1729	1926	1828	0.364
GE-1855	537.4	12.30	99.7	10.0	6.6	16.6	17.4	1655	1737	1696	0.443
GE-4398	594.6	11.95	99.3	9.5	8.2	17.8	17.5	1766	1738	1752	0.511
Mean (H)	541.3	11.12	97.6	10.7	7.1	17.7	17.9	1731	1746	1738	0.540
GE-2154	252.8	1.65	89.7	5.5	3.7	9.2	10.7	822	958	890	0.139
GE-2073	197.0	11.36	76.7	7.7	8.6	16.3	13.8	1249	1056	1152	0.116
GE-4201	133.5	9.85	96.3	9.1	5.2	14.3	17.4	1372	1680	1526	0.216
GE-3094	234.8	1.80	89.3	4.8	4.9	9.7	12.7	874	1131	1003	0.115
GE-3140	215.8	4.64	85.3	7.7	9.1	16.8	16.8	1426	1434	1430	0.346
GE-1376	489.6	8.97	98.3	8.0	8.8	16.7	18.4	1646	1810	1728	0.417

Continued....

TABLE 1 Continued....

Genotype	Grain yield (g m ⁻²)	PA	G (%)	RL (cm)	SL (cm)	TSL (cm)	Seed-ling DW	SVI-I	SVI-II	Mean SVI	DHA
GE-3767	256.0	0.84	89.3	5.6	6.0	11.6	11.1	1037	992	1015	0.195
GE-2644	225.3	10.21	99.3	8.1	7.3	15.4	19.3	1530	1916	1723	0.709
GE-2136	285.6	12.24	92.3	11.3	6.7	18.0	14.1	1669	1302	1485	0.414
GE-3764	253.5	6.94	99.3	8.3	5.7	14.0	16.2	1394	1605	1500	0.174
GE-3147	274.1	10.43	95.7	6.7	7.6	14.3	14.9	1363	1422	1392	0.318
GE-5116	198.9	9.43	93.7	7.3	5.8	13.2	15.6	1234	1460	1347	0.163
Mean (L)	251.4	7.36	92.1	7.5	6.6	14.1	15.1	1301	1397	1349	0.280
Sig. H & L		**	**	***	NS	**	**	**	**	**	**
Grand Mean		9.24	94.8	9.1	6.8	15.9	16.5	1516	1571	1544	0.406
SEm +		0.37	2.0	0.8	0.8	1.2	1.7	114	159	102	0.02
CD @ 5%		0.74	3.9	1.6	1.5	2.4	3.4	229	321	204	0.03
CV (%)		29.30	5.6	27.1	18.3	20.1	16.0	23	20	20	24.5

Footnote : PA : Phytic acid (mg/g); G% : Germination, RL : Root length (cm), SL : Shoot length (cm), TSL : Total seedling length (cm), Seedling dry weight (mg/seedling), DHA : Dehydrogenase activity (OD value)

2015) and 8.52 to 14.2 mg/g (Makokha *et al.*, 2002). The mean PA across genotypes was 9.24 mg/g (Table 1) which is relatively higher than the mean of 6.85 mg/g (Gunashree *et al.*, 2014) and 7.59 mg/g (Hiremath and Geetha, 2015) due to differences in genotypes used in the experiments (Tanaka *et al.*, 2015). The PA varies even with crop and grain colour, for instance, black rice has PA of 15.5 (low) to 35.4 mg/g (high) (Oo *et al.*, 2023). Contrastingly, white finger millet has a lower PA of 2.66 mg/g (Shigihalli *et al.*, 2018). In this regard, the processing techniques like soaking, germination, malting, roasting, boiling, popping, etc although decrease the anti-nutritional factors to increase the bioavailability of calcium, iron and zinc by reducing molar ratios of phytate: Ca/ Fe/ Zn (Bagade and Pant, 2022), identification of genotypes for LPA would be ideal. Interestingly, in normal brown finger millet, a very low PA of <1.8 mg/g was observed in the case of genotypes, GE-3767, GE-2154, and GE-3094 (Table 1). These LPA genotypes can be used in breeding approaches to reduce the PA in high-yielding popular varieties and to increase the bioavailability of micronutrients in humans (Sparvoli and Cominelli, 2015; Reddy *et al.*, 2022).

Seed Quality Parameters

The seed germination differed significantly between the genotypes. All the genotypes showed >86.0 per cent germination with an exception of the genotype GE-2073 having 76.7 per cent, which had a higher PA of 11.36 mg/g (Table 1). Relatively higher seed germination in HPA types could be due to release of PA-bound cations which act as osmoticum for the rapid uptake of water at the beginning of seed germination. The root length was higher than the shoot length, and a higher root length of 12.8 cm was observed in HPA genotypes, GE-4568, GE-1077, GE-199 and GPU-28 (12.4 cm), whereas, least root length was observed in LPA genotypes, GE-2154 (5.5 cm), GE-3094 (4.8 cm) and GE-3767 (5.6 cm). The total seedling length and seedling dry weight, respectively were also higher in HPA genotypes, GPU-28 (18.6 cm, 20.2 mg/seedling), GE-199 (20.2 cm, 22.4 mg/seedling), whereas lower seedling length and seedling dry weight were observed in GE-2154 (9.2 cm, 10.7 mg/seedling), GE-3094 (9.70 cm, 12.7 mg/seedling) and GE-3767 (11.6g, 11.1 mg/seedling). In the similar order, the mean seedling vigour index (SVI) was higher in HPA genotypes, GE-199 (2133), GPU-28 (1923), whereas least was observed in LPA

genotypes, GE-2154 (890), GE-3094 (1003) and GE-3767 (1015). The higher performance in seedling parameters in HPA types could be due to PA breakdown during seed germination that provides inorganic phosphorus, micronutrients (Zn^{2+} , $\text{Fe}^{2+/3+}$, Ca^{2+} , Mg^{2+} , Mn^{2+} and Cu^{2+}) and *myo*-inositol to the growing seedlings (Gupta *et al.*, 2015). Further, P increases the root hair number and length with up-regulation of EcPHT1:2 in roots (Maharajan *et al.*, 2023). In the present study, the high-yielding genotypes showed relatively higher SVI-I and SVI-II and the low-yielding genotypes GE-2154, GE-3767, GE-3094, GE-2073, showed substantially a lower seedling vigour index (Table 1). Earlier, a higher SVI-I and SVI-II reported to have a better phenotypic performance of seedling growth of major cereal, pulse, and oilseed crops under field conditions (Priya Reddy and Nanja Reddy, 2024). Low P in seeds is reported to decrease seedling vigour and crop establishment (Yugandhar *et al.*, 2022) due to increased ethylene production under P limited conditions (Oo *et al.*, 2023). The higher vigour in HPA types could be due to rapid root growth for better uptake of water and nutrients from the soil (Krishna and Reddy, 2021; Oo *et al.*, 2023).

The seed germination and seedling vigor mainly depend on the availability of seed reserves until the seedling becomes autotrophic. Breakdown of seed reserves into simple sugars is mediated by hydrolytic enzymes like dehydrogenase, amylases, proteases, lipases, and phytases (Guzman-Ortiz *et al.*, 2018). The catalysis of food reserves to produce glucose is mediated by a dehydrogenase (DHA) and translocates to the growing embryo (Hussain *et al.*, 2017; Bourioung *et al.*, 2020). In the present study, the DHA ranged from 0.115 to 0.891 with a mean of 0.406 OD (Table 1). A similar mean of 0.293 OD has been reported in wheat (Sharma *et al.*, 2017). The DHA was higher in high-yielding genotypes (0.540) compared to the low-yielding ones (0.280), thus high and low germination percentage and seedling growth, respectively (Table 1). Similar to these results, seed treatment with P (0.1%) has shown increased seed germination due to higher dehydrogenase activity (Bishnoi *et al.*, 1994; Bam *et al.*, 2006 and Sharma *et al.*, 2017).

Another important hydrolytic enzyme that influences the seed germination and seedling growth is the alpha amylase that breaks down the starch into glucose and transport to the embryo for the subsequent growth (Guzman-Ortiz *et al.*, 2018; Verma *et al.*, 2019). The alpha amylase enzyme activity was determined with (15 h) and without (0 h) hydration of seeds. The alpha amylase activity was higher upon hydration compared to without hydration of seeds. At the initial stages, without seed hydration, the GE-199 and GE-4568 of HPA genotypes did not differ significantly between each other, and the LPA genotypes, GE-3767 and GE-2154 also did not differ significantly, however, the HPA genotypes relatively had higher alpha amylase activity compared to LPA genotypes (Fig. 1). However, after imbibition for 15 hours, all the genotypes differed significantly irrespective of high or low PA genotypes. The de novo synthesis of alpha-amylase is induced by imbibition, and it was 61.2 per cent higher upon hydration for 15h compared to the dry seed (Fig. 1). Furthermore, the HPA genotypes had 10 per cent and 25.4 per cent higher alpha-amylase activity in dry and hydrated seeds, respectively, compared to the LPA genotypes (Fig. 1). It is evident that HPA genotypes inherently accumulate higher alpha amylase activity due to higher P available in the grain. Higher alpha-amylase activity in HPA

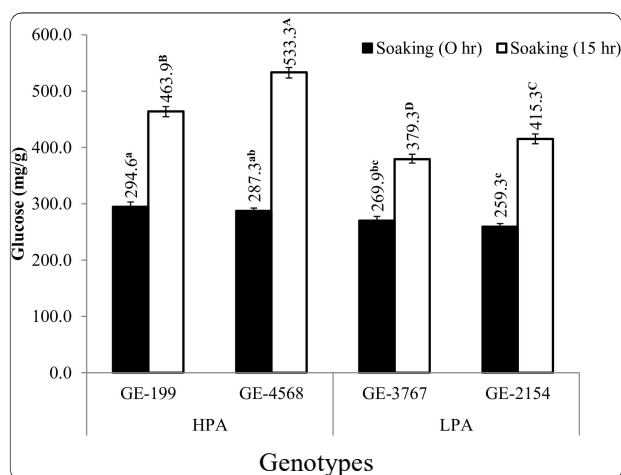


Fig. 1 : Alpha amylase activity (glucose production) in genotypes differing for grain phytic acid, measured before (0 hr) and after soaking in water (15 hr) and the lower and upper case letters indicates the comparison between genotypes before and after soaking, respectively.

genotypes could be due to higher P for ATP synthesis (Dong *et al.*, 2020; Padilha *et al.*, 2024) and higher calcium (Verma *et al.*, 2019; Padilha *et al.*, 2024). For instance, an increased P availability in the germination medium linearly increased the alpha-amylase and ATP production (Kikunaga *et al.*, 1991). Therefore, HPA types are suitable for higher seed germination and seedling vigour, and reiterates the importance of accumulation of PA in plant's perspective in contrast to human perspective.

Relationship between Phytic acid, Seedling Vigour, and Grain Yield

Under adequate soil P, seeds with low or high P perform similar (Rose and Raymond, 2020). However, most soils are low in P (Sanyal *et al.*, 2015), and hence a higher seed P is essential to achieve a higher seedling vigor. The higher seed germination in HPA genotypes could be ascribed to a higher DHA with a correlation of 0.584** (Table 2). In this direction, the field emergence of finger millet varieties was found positively and significantly correlated to the seed germination percent, seedling length (root and shoot), seedling dry weight and seedling vigour indices (Shanthala *et al.*, 2013; Sharma *et al.*, 2017 and Rawat *et al.*, 2023) and even the soil DHA was also linked to soil P (Gaind *et al.*, 2006). Further, the DHA was positively related to PA ($r = 0.544^{**}$; Table 2) indicating the relevance of DHA on seed germination.

This is the first documentation about PA and DHA in finger millet.

Furthermore, the PA showed a positive and significant relationship with mean SVI ($r = 0.715^{**}$) and grain yield ($r = 0.431^{*}$; Fig. 2; Oo *et al.*, 2023), suggesting that the seed PA has a positive role in plant perspective. Hence, seedling vigor and or seed PA can be used as a surrogate for the preliminary screening of a large number of genotypes. Furthermore, the positive relationship between the SVI and grain yield ($r = 0.628^{**}$; Fig. 2) reiterates that the PA is essential for seedling vigor and grain yield under rainfed conditions. Similarly, LPA mutants have been identified in maize and barley (Lott *et al.*, 2000; Colombo *et al.*, 2022) and even the LPA mutants have shown a better root and shoot length of 14d old seedlings (Colombo *et al.*, 2022). Therefore, identifying the low PA genotypes without much affecting the seedling vigor would be an ideal strategy for higher bioavailability of essential micronutrients (iron and zinc) in humans (Reddy *et al.*, 2022). In comparison to the popular variety, GPU-28, the LPA genotypes had very lower PA by 88.2% (GE-2154), 87.2% (GE-3094) and 94.0% (GE-3767), whereas, the SVI was by 53.7% (GE-2154), 47.9% (GE-3094), and 47.2% (GE-3767; Fig. 3) can be used as donors to reduce the grain PA content of popular varieties, to improve the bioavailability of

TABLE 2

Pearson correlations between grain phytic acid and seedling parameters in finger millet genotypes

Parameter	Phytic acid	G (%)	Root length	Shoot length	Seedling length	Seedling dry weight	Mean SVI
Grain Phytic acid	1.000						
Germination %	0.395 *	1.000					
Root length (cm)	0.687 **	0.503 **	1.000				
Shoot length (cm)	0.447 *	-0.097	0.195	1.000			
Seedling length	0.757 **	0.372 *	0.907 **	0.589 **	1.000		
Seedling dry weight	0.623 **	0.574 **	0.709 **	0.384 *	0.749 **	1.000	
Mean Seedling vigour index	0.715 **	0.700 **	0.861 **	0.392 *	0.877 **	0.936 **	1.000
Dehydrogenase activity (DHA)	0.544 **	0.584 **	0.817 **	0.288	0.797 **	0.729 **	0.848 **

Note: * and **, respectively significant at 5 and 1% level.

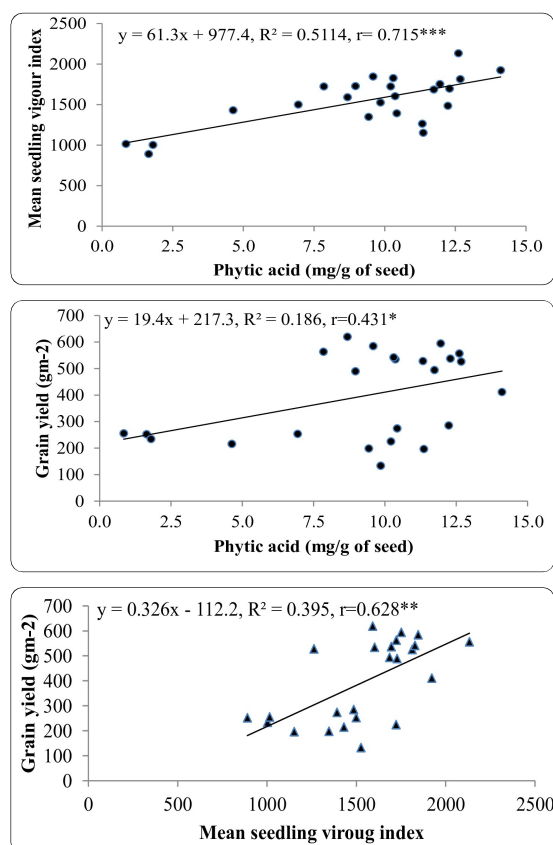


Fig. 2: Relationship between grain phytic acid, seedling vigour index and grain yield in finger millet genotypes

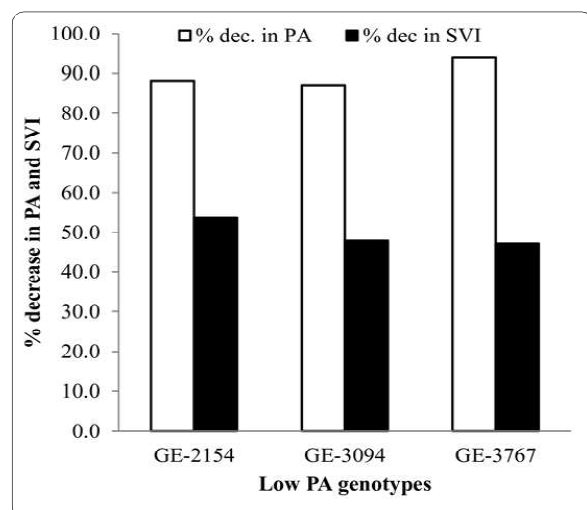


Fig. 3: Comparison of low phytic acid genotypes with popular variety GPU-28 for percent decrease in phytic acid content and seedling vigour index in finger millet

micronutrients in humans. To support this only a 10 per cent lesser in grain yield was reported in LPA (5.71 mg/g) genotype against a high PA genotype with 7.57

mg/g of finger millet (Reddy *et al.*, 2022). Therefore, the identified genotypes (GE-3767, GE-2154 and GE-3094) with LPA of <1.8 mg/g could be used to reduce the grain PA content of popular varieties.

From plant perspective, accumulating a higher grain PA is an evolutionary mechanism for better seedling survival and perpetuation. Contrastingly, in human perspective, seeds with low PA are suitable for better bioavailability of essential micronutrients like iron and zinc. Therefore, it would be apt to select genotypes having lower seed PA with higher seedling vigour, and such genotypes identified in the present study can be utilized in breeding approaches to decrease grain PA of popular varieties.

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