Mineral constituents in seeds of horsegram mutants

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ABSTRACT

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) seeds containing macro nutrients like N, P, K and micronutrients like Mg, Zn, Cu, Fe and Mn are the major plant nutrients involved in various physiological, biochemical and enzymological processes. The growth, development and various metabolic functions like photosynthesis, carbohydrate and protein synthesis etc. mainly depend on plant nutrient balance. Considering this the mineral constituents in seeds of viable mutants was investigated. The seeds of eight mutants were analysed for minerals such as N, P, K, Mg, Zn, Cu, Fe,Mn. The results have revealed changes in the concentrations of macro and micro mineral elements in the mutant seed material. These changes in concentrations are attributed to the altered genetic structure, due to mutations.

Keywords : Horsegram, minerals , mutant, nutrients

INTRODUCTION

In post-green revolution era, the per capita availability of pulses has sharply declined in the country. In spite of the largest area under chickpea, pigeonpea, lentil and dry beans. India's position in average production is not good. Except chickpea and pigeonpea, the average production of other pulses including horsegram in the country is significantly lowered than the average yield in the world. The productivity level of almost all pulses is very low in India.

The major constraints for this are: Lack of high yielding varieties adapting to diverse agroclimatic conditions. Large area under rainfed cultivation (88%). Biotic and abiotic stress conditions caused up to 30% losses. Poor plant stand. Poor response to high input conditions and very poor crop management practices. Moisture stress generally at the terminal growth phase and inadequate seed replacement rate and non availability of quality seeds. Deficiencies of major as well as micronutrients, low risk bearing capacity and resource poor farmers.

On the technological front, pulses still need major breakthrough in yield levels, through morphophysiological changes in plant type and development of multiple disease resistant varieties, coupled with tolerance to abiotic stresses. Inadequate or no transfer of technologies, poor storage, lack of processing and marketing facilities further discourage farmers to extend areas under pulse production [1].

Pulses being rich source of protein, minerals, vitamins and crude fiber, they are considered as health food, which offer nutritional security to millions of people, suffering from protein malnutrition, especially in South Asia and Africa [2]. They are valued as relatively cheaper source of protein and energy as compared to animal proteins. Legumes also contain several nutritional factors whose beneficial effects on human health need to be fully exploited [3]. Horsegram is the important source of protein, iron and molybdenum and minerals including Na, K, Ca, Mg, S, P, and Cl as well as the micro-minerals, Fe, Zn, Cu, Mn, I, F, Se, Mo, Co and essential for human life [4, 5 and 6].

MATERIALS AND METHODS

The authentic seeds of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) cultivar Dapoli Kulthi-1 were procured from Department of Botany, Dr. Balasaheb Savant Konkan Krishi Vidyapeeth, Dapoli, Dist-Ratnagiri (M.S.). Gamma rays, ethyl methane sulphonate (EMS) and their combinations were employed in present study for the treatments of seeds of horsegram Dapoli Kulthi-1.

To begin with, pilot experiments were conducted to determine the lethal dose (LD_{50}), suitable concentrations of EMS and duration of seed treatment. The doses of gamma rays, 100 to 400Gy and EMS 0.2% to 0.5% were finally selected for the seed treatment and the duration fixed was four hours. Selected seeds were soaked in distilled water for 10 hours and the wet seeds were treated with different concentrations of EMS (0.2, 0.3, 0.4 and 0.5 % v/v) for four hours. For combination treatments the gamma irradiated seeds were treated with different concentrations of EMS. The untreated seeds served as control. The seeds treated with various concentrations of EMS were washed thoroughly with tap water for two hours to terminate the reaction of chemical mutagen and to leach out the residual chemicals. The lot of treated seeds (675) from each treatment was used for raising M_1 generation in field fallowed by M_2 and M_3.

Present investigation was carried out at Department of Botany, University of Pune, Pune- 411 007 (M.S.). The soil type of the experimental field was slightly deep, fine and calcareous with good drainage. The average minimum temperature was recorded as 17.63^{0}C and maximum 32.73^{0}C with average annual rainfall 641.03mm.

The field experiments were conducted on the experimental field. The crop of horsegram Dapoli Kulti-1 was grown in *Kharif* season under uniform conditions. All the experiments were carried out in triplicate following RBD design. The size of each (treatment) plot was 3.75m X 2.75m and each plot had 225 plants. The distance between two rows and two plants was 30 X 15 cm and the distance between two adjacent plots was one meter. Seeds of M_2 mutants and control were sown in field with randomized block design replicated thrice. 675 seeds of each treatment were used to raise M_3 generation.

Mineral Analysis

Dried horsegram seed samples were ground to a fine powder using grinder. Three samples (0.25 g each) from each ground sample were digested and processed for mineral analysis. Samples (0.25g) with 3 mL ultra-pure nitric acid were taken in glass tubes for pre-digestion overnight. After 12 hours tubes were kept in a digestion block (Magnum Series; Martin Machine, Ivesdale, IL, USA) at 125°C for four h (with refluxing). Then, tubes were removed and cooled for 5 min. 2 mL of hydrogen peroxide was added and kept in digestion block at 125°C for 1 h. This hydrogen peroxide procedure was repeated twice. The digestion block temperature was raised to 200°C until they were dry. After removal from the block cooled and digested samples were resuspended in 2% ultra-pure nitric acid overnight, then vortexed and transferred to plastic storage tubes until analysis for Cu, Fe, K, Mg, Mn, P, and Zn concentrations. Mineral analysis was performed using ICP-OES (inductively coupled plasma-optical emission spectroscopy). Seed mineral concentrations were determined on a dry weight basis [7].

Statistical analysis

The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at $p= 0.05$. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.3.5).

RESULTS

Mineral constituents in seeds of M_3 mutants

Nitrogen: Nitrogen content in seeds of viable mutants ranged from 0.52 to 0.39% (Table-1). All the mutants except compact showed increase in N content over control. Highest content of nitrogen was found in high yielding (0.52%), but the compact mutant showed lower nitrogen content (0.39%) over control (0.41%).

Nitrogen has predominant role in plants, being the constituent of proteins, nucleic acids, nucleotides, phytohormones etc. Not only this, but it also regulates photosynthesis, water use efficiency, NR activity, carbohydrate metabolism, biosynthesis of pigments and many secondary metabolites. It also governs growth, canopy development, productivity and yield in crop plants. Increased nitrogen content (Table-1) was recorded in seeds of high yielding and early mutants which might have contributing above mentioned metabolic activities of the mutants of horsegram. The total nitrogen content was positively correlated with nitrate reductase activity [8, 9 and 10].

Phosphorus: All mutants except compact, high yielding and early showed decrease in phosphorus content than control (Table- 1). Phosphorus is an indispensible element for plant growth, its concentration in terrestrial plant is 0.2%. Increase or decrease in P content and is closely related with carbohydrate and starch metabolism [11]. The rate of plant growth and level of metabolic activities are also dependent on P content.

In seeds the phosphorus content was decreased in dwarf mutant. Similar results have been reported by [12, 13, 14 and 15]. Reduced phosphorus content in seeds may reduce the phytate level, which is known as a strong antinutritional factor and as inhibitor for seed germination.

Potassium: Potassium content of viable mutants ranged from 0.89% to 0.67% (Table-1). Improvement in K contents was observed in the compact (0.89%) and dwarf (0.85%) mutants over control (0.84%). All the remaining mutants have lower in K than control (Table-1). Potassium is highly mobile, helping in translocation of carbohydrates, regulates the opening of stomata and maintains cellular organization and cell permeability. It plays a vital role in activation of many enzymes involved in photosynthesis, protein synthesis and respiration. It is also involved in increasing leaf area index, CO_2 assimilation rate, stimulation of the translocation of photo assimilates, and increasing crop productivity. Hence the increased K might have directly or indirectly contribute in above metabolic processes of horsegram mutants.

Magnesium: The results shown in Table-1 revealed that all the mutants except gigas and late showed positive influence on magnesium contents. Maximum contents of magnesium were found in high yielding and compact (0.19ppm), while minimum was recorded in gigas and late (0.14ppm) mutants over control (0.15ppm).

The concentration of Mg in glycophytes is 0.2% on dry tissue basis, which acts as a cofactor in enzymatic synthesis of sucrose and plays important role in carbohydrate, protein as well as nucleic acid metabolism[16]. It also regulates photophosphorylation, carboxylation and nutrient uptake [17]. The enhanced level of Mg was responsible for increasing the plant height, leaf area, yield, photosynthetic rate, carbohydrate and protein contents of mungbean, chickpea and fenugreek [18]. Increase in chlorophylls to increased Mg level [19]. The magnesium content was increased in seeds of tall, dwarf, compact, high yielding, early and long pod mutants than control (Table-1).

Zinc: Results recorded in Table- 1 indicated that all mutants showed increase in zinc content over control. Highest content was found in high yielding (34.67ppm), but lowest (27.04ppm) was noted in compact mutants than control (27.10ppm).

It is essential for the activity of several enzymes, transformation of carbohydrates, synthesis and maintenance of auxin balance. Zinc deficiency retards photosynthesis [20]. Its active participation in biosynthesis of chlorophylls, proteins and axuins is well documented. Zinc is also important in starch synthesis and other metabolic processes. The results depicted in Table-1showed that there was decrease in zinc content in seeds of some mutants such as compact, however there was increase in gigas, compact, early tall, dwarf, late and long pod mutants which might have plays a positive role in above mentioned metabolic processes.

Copper: The results noted in Table-1 illustrated that all the mutants except tall and late showed increase in copper content. Highest contents of copper were recorded in high yielding (23.31ppm), while minimum was noted in tall (19.12ppm) mutants over control (20.34ppm).

Table: 1 Mineral constituent in seeds of horsegram mutants cv. Dapoli-Kulthi-1.

Mutants	N (%)	P (%)	K (%)	Mg (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
Control	0.41	0.27	0.84	0.15	27.10	20.34	213.21	96.05
Gigas	0.48	0.27	0.75	0.14	28.34	21.56	217.15	112.16
Tall	0.44	0.24	0.79	0.18	28.22	19.12	221.56	91.22
Dwarf	0.41	0.25	0.85	0.17	29.55	21.25	210.34	89.47
Compact	0.39	0.29	0.89	0.19	27.04	22.40	224.59	109.34
High yielding	0.52	0.28	0.67	0.19	34.67	23.31	215.78	110.63
Early	0.49	0.28	0.72	0.17	28.56	22.54	226.48	97.39
Late	0.43	0.21	0.78	0.13	28.32	19.47	219.30	95.11
Long pod	0.46	0.26	0.81	0.17	32.15	23.20	214.48	107.67

It is an important component of photosynthetic electron transport system. Copper is translocated in plants in the form of amino complexes and most of the Cu remains in association with low molecular weight soluble proteins in leaves [21] . The results recorded in Table-1reveled that the copper level was decreased in seeds of tall and late mutants as compared to control. The processes depending on Cu as cofactors may have positive or negative impact on particular mutants of horsegram.

Iron: The results presented in Table-1 illustrated that the mutants except dwarf showed increase in iron contents over control. The iron contents of viable mutants ranged from 226.48ppm to 210.34ppm. Maximum contents of iron were found in early (226.48ppm), while minimum was recorded in dwarf (210.34ppm) mutants over control (213.21ppm).

The average value of iron in higher plants is a 2 µmole g^{-1} of dry tissue [16]. It is essential component of the enzyme systems like catalase, peroxidase, cytochrome oxidase and ferredoxin .The deficiency of iron causes decrease in protein content and an increase in the level of soluble amino acids. [11] reported that Fe is essential for many vital metabolic functions in plants e.g. chlorophyll synthesis, enzymatic reactions, respiration, photosynthesis etc. The role of iron in metabolic processes like cell division, PS I, PS II, photosynthetic activity, fruit development and yield is well documented by [22, 18, 18 and 23]. The identification of mutants with high amounts of Fe and Zn could contribute significantly to improve the micronutrient status of the diet of consumers.

Manganese: The results recorded in Table-1 illustrated that all mutants except dwarf showed positive impact on manganese contents. Manganese acts as catalyst in many important enzymatic and physiological reactions in plants. It plays an important role in respiratory process of plants is involved I oxidation of carbohydrate. It activates the enzymes associated with the metabolism of nitrogen and synthesis of chlorophyll. The results presented in (Table-1). illustrated that the mutants such as high yielding, compact, long pod and gigas had shown positive impact on manganese, but tall, dwarf and late mutants showed negative impact on manganese content over control.

CONCLUSION

The macro and micro plant nutrients are involved in various physiological, biochemical and enzymological processes. The growth, development and various metabolic functions like photosynthesis, carbohydrate and protein synthesis etc. mainly depend on plant nutrient balance. The results will also help directly or indirectly to interpret the changes induced in various metabolic processes and enzymological activities of M_3 plants. The nutritionally superior mutants will be a promising material for plant breeders in feature to make the improved and superior quality varieties for rainfed area of the Maharashtra state.

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