American Journal of Food Technology 7 (7): 437-444, 2012
ISSN 1557-4571 / DOI: 10.3923/ajft.2012.437.444

Variability and Correlations Studies for Total Iron and Manganese Contents of Chickpea (*Cicer arietinum* L.) High Yielding Mutants

[1]Mohammad Imran Kozgar, [1]Samiullah Khan and [2]Mohd Rafiq Wani
[1]Department of Botany, Mutation Breeding Laboratory, Aligarh Muslim University, Aligarh-202 002, U.P., India
[2]Department of Botany, Government Degree College, Anantnag-192 231, J and K, India

Corresponding Author: Mohammad Imran Kozgar, Department of Botany, Mutation Breeding Laboratory, Aligarh Muslim University, Aligarh-202 002, U.P., India

ABSTRACT
Increased malnutrition and food insecurity for the past several years in countries, like India, is point of great concern. Present study was undertaken to reveal the potential role of mutagenesis to induce genetic variability of total seed Fe (μg g^{-1}) and Mn (μg g^{-1}) contents and their correlations to the yield/plant (g) in chickpea. Fe and Mn deficiencies are usually responsible for major malnutrition conditions, while lower yield of crops results to food insecurity. Seeds of chickpea were treated with ethylmethane sulphonate (EMS), gamma rays and their combinations and studied their effects on the components of variation for yield/plant, the total seed Fe and Mn contents. Six high yielding mutants were isolated in M_2 generation and their Fe and Mn contents were evaluated. Positive correlation was seen between total Fe and Mn contents to yield in the isolated mutants at lower doses of mutagen while the correlation between Fe to Mn contents was found to be negative.

Key words: Mutagenesis, trace elements, yield-correlation, chickpea

INTRODUCTION
According to the National Family Health Survey 3 (NFHS 3, 2006a), despite the fact that India's economy is 10th largest whereas that of sub-Sahara is on 79th position in world economic market, this country is in one of the most critical indices where the malnutrition conditions has jumped to 42.5% as compared to 35% of sub-Sahara (NFHS 3, 2006a). The anemic conditions has also increased nearly about 9% from 1998-99 to 2005-06 (NFHS 3, 2006b), pooled for all aged groups. The induction of mutations in the major pulse crops for generating high yielding mutants (Yaqoob and Rashid, 2001; Addai and Safo Kantanka, 2006; Goyal and Khan, 2010; Joshi *et al.*, 2011; El-Sayed *et al.*, 2012) and assessing the genetic variability for their trace elements could be a novel strategy to combat these concurrent problems in Indian regions. The cultivation of chickpea (*Cicer arietinum* L.) in the northern regions of India has decreased drastically from about 4.7 to only 0.9 million during the period from 1965-1966 to 2005-2006 and in Madhya Pradesh, it is almost now non-existent and henceforth, the rich source of iron is losing in the particular regions (Singh, 2010). This increased the anemic conditions and increase in health related problems in addition to food insecurity. Reports for positive correlation of total trace elements in seeds, like Fe and Mn, to yield in chickpea are limited and the role of induced mutagenesis in this area could be novel to isolate desirable breeding lines of varieties of crops for desired characters.

Mutation breeding has become a proven way of creating variation within a crop variety and offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution (Novak and Brunner, 1992; Baloch *et al*., 2003). Mutation breeding combines several advantages in plant improvement by upgrading a specific character without altering the original genetic make-up of the cultivar and is a well functioning branch of plant breeding, supplementing to conventional methods in a favourable manner (Toker *et al*., 2007; Srivastava *et al*., 2011; Mostafa, 2011). In contrast transposon or T-DNA insertional mutagenesis generally leads to complete disruption of gene function rather than in generating allelic series of mutants with partial loss of function and thus not producing the range of mutation strengths necessary for crop improvement (Chopra, 2005; Parry *et al*., 2009). In addition the insertion sites within the genome may not be distributed randomly (Zhang *et al*., 2007) and hence increasing the number of insertion lines required for full genome coverage to unrealistic level (Parry *et al*., 2009). However, chemical and physical mutagenesis have a number of advantages over such approaches as mutagens introduce random changes throughout the genomes, generating a wide range of mutations in all target genes and a single plant can contain a large number of different mutations resulting a manageable population sizes (Parry *et al*., 2009).

In order to break food insecurity and malnutrition scenario in developing countries like India, efforts are needed to develop high yielding and mineral enriched varieties of crops like chickpea with appropriate growth habit. Creation of genetic variability followed by screening and selection of the best plants is a major target for this crop. The possibility offered by mutagenic agents to induce new genetic variation is, therefore, of extreme interest and importance. Since, chickpea is a self-pollinated crop, mutation breeding could be rewarding for broadening the genetic base of total plant yield, yield contributing traits and other important traits like nutrition compositions. In the present study success rate of enriching high yielding chickpea with mineral elements like Fe and Mn through induced/artificial mutagenesis have been tried out and that could be rewarding once successfully screened for further generations for character stabilization for the traits in concern.

MATERIALS AND METHODS

Procurement and characters of biological material: Seeds of desi chickpea variety Pusa-256 procured from Government Seed Store, Aligarh, Uttar Pradesh (India), This chickpea variety have bold seeds having wide adaptability released from Pusa, Indian Agricultural Research Institute, New Delhi with pedigree (JG 62×850-3/27)×(L550×H208).

Mutagenic treatments and experimental design: Uniform and healthy seeds cleaned with distilled water were treated with: (1) four doses of Gamma rays (100, 200, 300, 400 Gy) from radioisotope ^{60}Co source at the National Botanical Research Institute, Lucknow, India, (2) Four concentrations (0.1, 0.2, 0.3, 0.4%) of ethylmethane sulphonate, EMS (an alkylating agent manufactured by Sissco Research Laboratories Pvt. Ltd., Mumbai, India) for 6 h, after 9 h presoaking in distilled water and (3) their combinations (100 Gy+0.1, 100 Gy+0.2, 200 Gy+0.1, 200 Gy+0.2%), first by gamma rays and then directly treated in respective concentration of EMS solution for 6 h. Solutions of chemical mutagen, used alone and in combinations were prepared in phosphate buffer of pH 7. For each treatment three hundred seeds were used. Treated seeds were sown in the field, in Rabi growing seasons of October 2008, with three replications in a randomized complete block design, with each replication consisting of 100 seeds. Seeds soaked in distilled water were used as controls. Seeds of M_1 plants and control plants of chickpea were harvested separately and sown in plant progeny rows to raise M_2 generation in October 2009. High yielding mutants were isolated and their total seed Fe and Mn contents were evaluated.

Analysis of total seed Fe and Mn contents: Seeds of high yielding mutants (at moisture content of 12%) were digested by wet diacid methodology (Gupta, 2004) and Fe and Mn contents were determined by the Atomic Absorption Spectrophotometer, AAS (Part No. 01-0818-01, GBC Scientific Equipment Pty Ltd., Victoria, Australia) using a respective standard from MERCK manufactures, Mumbai for calibration. The cathode lamp used has been set up at the slit width of 0.2 nm for the Fe and Mn contents estimation.

Statistical analysis: Statistical data for total Fe and Mn contents were assessed by computer software AVANTA 2.0 version compatible to AAS computer equipments. Components of variation (Phenotypic coefficient of variation (PCV), Genotypic co-efficient of variation (GCV), Heritability in broad sense (h^2) and Genetic advance as percent of mean (GA% of $\bar{X}$) for total seed Fe content, total Mn content and for yield/plant in isolated high yielding mutants were calculated by the formulae suggested by Singh and Chaudhry (1985), Johnson *et al.* (1955) and Allard (1960) as outlined and adopted by Wani an d Khan (2006). The correlation studies were done by the methodologies given by Hoshmand (1998).

RESULTS AND DISCUSSION

Induction of mutation in chickpea through chemical and physical mutagens has been investigated for long time and many reports have been presented for creating the morphological and physiological variations by affecting growth habit, flower colour and plant type etc. for the selection of high yielding mutants in early generations (Basu, 1967; Khan *et al.*, 2004; Tah, 2006; Deniz, 2007; Kozgar and Khan, 2009; Kozgar *et al.*, 2011; Nakagawa *et al.*, 2011; Mostafa and Alfrmawy, 2011) but assessing the impact of mutagens on the trace elements like that of Fe and/or Mn and their genetic variation has not been extensively studied. However, the fact is that, plant breeding needs genetic variation of useful traits for crop improvement (Novak and Brunner, 1992; Alsemaan *et al.*, 2011). Present investigation has shown that the total seed Fe and Mn content has increased in the isolated high yielding mutant and in coherence with the values of yield/plant (Table 1-3). Results obtained in present study are in conformity to the

Table 1: Estimates of mean values ($\bar{x}$) and genetic parameters of total yield (g) in high yielding isolates in M_2 generation of chickpea var. Pusa-256

Treatment	Mean±SE	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\bar{x}$)
Control	26.61±1.18[c]	16.54	8.48	26.34	11.47
Gamma rays					
100 Gy	31.03±0.57[ab]	20.35	18.15	79.55	42.69
200 Gy	30.70±0.68[ab]	21.76	17.95	68.10	39.07
EMS					
0.1%	31.22±1.12[ab]	20.19	18.01	79.53	42.34
0.2%	33.42±0.55[a]	21.64	19.86	84.55	48.24
Gamma rays+EMS					
100 Gy+0.1%	32.44±0.78[ab]	20.82	19.56	88.34	48.43
200 Gy+0.1%	29.78±0.98[b]	19.23	17.54	83.42	42.25

Values with different alphabetical letters are significant at 5% level calculated by SPSS 16.0 computer software by Duncan's multiple range test

Table 2: Estimates of mean values ($\bar{x}$) and genetic parameters of total seed Fe content ($\mu g\ g^{-1}$) in high yielding isolates in M_2 generation of chickpea var. Pusa-256

Treatment	Mean±SE	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\bar{x}$)
Control	70.0±2.11^c	3.78	1.72	20.72	2.07
Gamma rays					
100 Gy	76.2±1.17^b	7.49	5.36	51.27	10.13
200 Gy	74.5±1.41^b	9.44	6.28	44.25	11.02
EMS					
0.1%	76.7±1.19^b	7.93	5.50	48.05	10.06
0.2%	76.0±0.89^b	7.78	5.66	52.98	10.87
Gamma rays+EMS					
100 Gy+0.1%	82.1±0.97^a	10.94	7.30	45.51	12.85
200 Gy+0.2%	78.0±1.07^b	9.21	5.93	41.48	10.08

Values with different alphabetical letters are significant at 5% level calculated by SPSS 16.0 computer software by Duncan's multiple range test

Table 3: Estimates of mean values ($\bar{x}$) and genetic parameters of total seed Mn content ($\mu g\ g^{-1}$) in high yielding isolates in M_2 generation of chickpea var. Pusa-256

Treatment	Mean±SE	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\bar{x}$)
Control	30.32±0.53^{cd}	3.51	1.62	21.66	2.03
Gamma rays					
100 Gy	31.56±0.78^{bcd}	6.55	4.56	49.11	8.45
200 Gy	30.45±1.37^{cd}	7.60	5.17	46.51	9.30
EMS					
0.1%	33.45±1.28^{ab}	7.53	5.02	45.14	8.94
0.2%	34.23±0.46^a	7.66	5.29	47.24	9.53
Gamma rays+EMS					
100 Gy+0.1%	32.34±0.50^{abc}	6.37	4.34	45.64	7.67
200 Gy+0.2%	29.34±0.60^e	6.08	4.10	45.67	7.35

Values with different alphabetical letters are significant at 5% level calculated by SPSS 16.0 computer software by Duncan's multiple range test

results obtained under the Consultative Group on International Agriculture Research Micronutrient Project (CGIARMP) which hypothesized that it is possible to combine the high micronutrient trait with high yield, unlike protein content and yield that are negatively correlated through breeding strategies (Gregorio, 2002). Present findings have also confirmed the positive correlation in early generation between total seed Fe and Mn content to yield/plant in isolated mutants, respectively (Fig. 1, 2). The correlation between the yield/plant and the total seed Fe content showed decreasing trend with the increase in mutagen dose, in fact in combination treatment of gamma rays and EMS (200 Gy+0.1%) the relation was negative indicating that the lower doses could be a pivotal role in creating positive correlation between Fe and yield content. Similar trend were assessed in the correlation studies of total Mn content and yield/plant, however, the negative results were not obtained at any dose in this case (Fig. 2). The correlations between Fe and Mn were also seen to be positively correlated but at lower dose (Fig. 3). The estimates of PCV were higher than the GCV indicating that the environment variation was also significant in both the traits studied. Highest estimation of GCV for yield/plant (19.86%), total seed Fe content (17.23) and total Mn content (34.23) was recorded at 0.2% of EMS (Table 1), 100 Gy+0.1% EMS (Table 2) and 0.2% of EMS (Table 3), respectively. Mean value of yield/plant in isolated mutants were increased form 26.62 to 33.42 g (Table 1), while that of Fe and Mn contents increased from

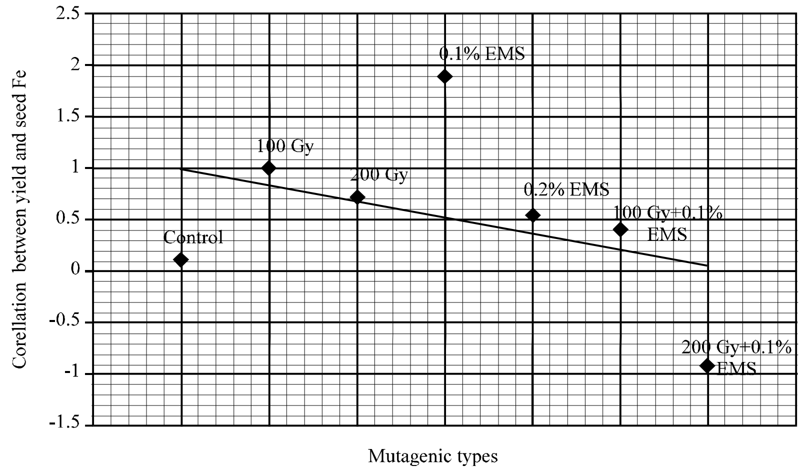

Fig. 1: Correlation between yield per plant (g) and total seed iron (Fe) content (μg g^{-1}) in the chickpea high yielding mutant isolate in different mutagenic treatments

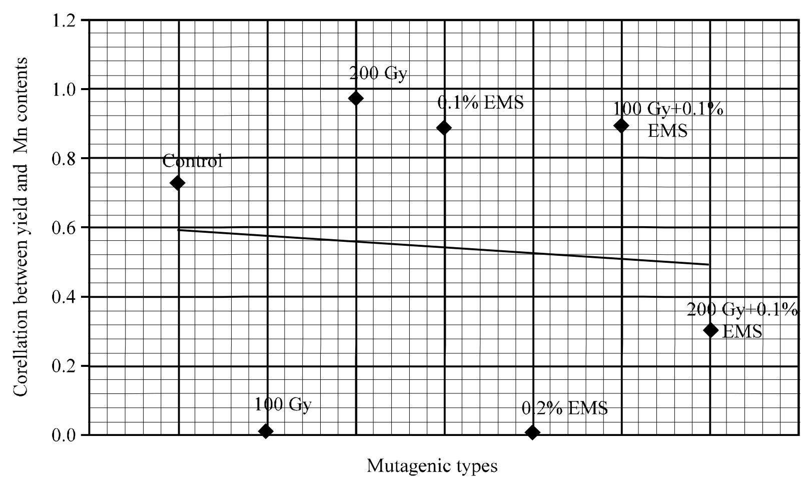

Fig. 2: Correlation between yield per plant (g) and total seed manganese (Mn) content (μg g^{-1}) in the chickpea high yielding mutant isolate in different mutagenic treatments

70.0 to 80.1 μg g^{-1} (Table 2) and 30.32 to 34.32 μg g^{-1} (Table 3), respectively. Increase in mean values and genetic variability for yield/plant and total seed Fe and Mn content of the isolated mutant lines from that of control values is an indication of the wider scope for genetic improvement of this crop in breeding programmes, even for trace elements. High heritability and along with high genetic advance was recorded for all yield/plant, total seed Fe and Mn content with respect to the control values indicating that these traits are governed by additive gene action and continued selection in subsequent generations will be highly responsive. The increase in heritability is indication of effective selection and more useful when coupled with genetic advance (Johnson *et al.*, 1955). Correlations among the traits studied were found to be of different values and directions. This might be due to the agonistic and antagonistic metabolic pathways followed (Kabata-Pendias, 2011) which leads to differential response in terms of correlation output values.

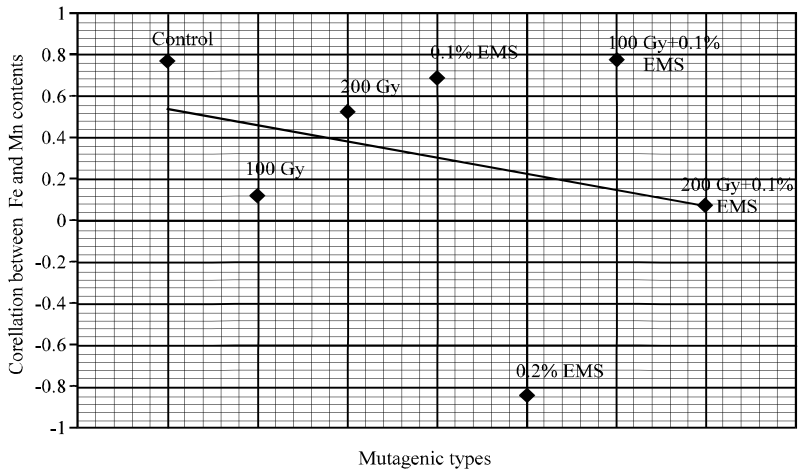

Fig. 3: Correlation between total seed iron (Fe) (μg g^{-1}) and total seed manganese (Mn) content (μg g^{-1}) in the chickpea high yielding mutant isolate in different mutagenic treat

CONCLUSION

As per the present findings, data showed that the total seed Fe and Mn composition of chickpea seeds in the high yielding mutants offer to the nutritionalists the possibility of using the mutation breeding an excellent source for creating variability of trace elements and is going to be a novel tool to combat malnutrition in addition to rescue from the food insecurity conditions on long term future goals, once and all handled in sustainable manner.

ACKNOWLEDGMENTS

We are grateful to the Chairman, Department of Botany, Aligarh Muslin University, Aligarh for providing the research facilities to carry out the present work and first author is also thankful to UGC, Government of India, for providing financial support in the form of Research Fellowship (BSR-UGC).

REFERENCES

Addai, I.K. and O. Safo-Kantanka, 2006. Effect of ^{60}Co gamma irradiation on storability of soybean seed. Asian J. Plant Sci., 5: 221-225.

Allard, R.W., 1960. Principles of Plant Breeding. 1st Edn., John Wiley and Sons Inc., New York.

Alsemaan, T., N. Albatal, H. Baydar and K. Almaarri, 2011. Genetic diversity and qualitative variation of *Rosa damascene* in Syria. Int. J. Agric. Res., 6: 429-436.

Baloch, A.W., A.M. Soomro, M.A. Javed, H.R. Bughio and S.M. Alam *et al*., 2003. Induction of salt tolerance in rice through mutation breeding. Asian J. Plant Sci., 2: 273-276.

Basu, R.K., 1967. Induction of high-yielding mutants in jute. Mutation Res., 4: 163-167.

Chopra, V., 2005. Mutagenesis: Investigating the process and processing the outcome for crop improvement. Curr. Sci., 89: 353-359.

Deniz, B., 2007. Selection for yield and earliness in mutated genotypes of spring barley (*Hordeum vulgare*) in cool and short-season environments. New Zealand J. Crop Horticul. Sci., 35: 441-227.

El-Sayed, H.E., S.A. Mahfouze, A.D. Shaltout, K.A. El-Dougdoug and R.A. Sayed, 2012. Chemical mutation induction *In vitro* cultured shoot tip of banana cv. Grand nain for resistance some virus diseases. Int. J. Virolo., 8: 178-190.

Goyal, S. and S. Khan, 2010. Induced mutagenesis in urdbean (*Vigna mungo* L. Hepper): A review. Int. J. Bot., 6: 194-206.

Gregorio, G.B., 2002. Progress in breeding for trace minerals in staple crops. J. Nutr., 132: 500S-502S.

Gupta, P.K., 2004. Soil, Plant, Water and Fertilizer Analysis. 2nd Edn., Agrobios, India.

Hoshmand, A.R., 1998. Statistical Methods for Environmental and Agricultural Sciences. 2nd Edn., CRC Press, Boca Raton, New York, USA.

Johnson, H.W., H.F. Robinson and R.E. Comstock, 1955. Estimates of genetic and environmental variability in soybeans. Agron. J., 47: 314-318.

Joshi, N., A. Ravindran and V. Mahajan, 2011. Investigations on chemical mutagen sensitivity in onion (*Allium cepa* L.). Int. J. Bot., 7: 243-248.

Kabata-Pendias, A., 2011. Trace Elements in Soil and Plants. 4th Edn., CRC Press, Boca Raton, FL USA.

Khan, S., M.R Wani, M. Bhat and K. Parveen, 2004. Induction of morphological mutants in chickpea. ICPN, 11: 6-7.

Kozgar, M.I. and S. Khan, 2009. Genetic improvement of chickpea through induced mutation. J. Phytol., 1: 422-424.

Kozgar, M.I., S. Goyal and S. Khan, 2011. EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*. Res. J. Bot., 6: 31-37.

Mostafa, G.G. and A.M. Alfrmawy, 2011. RAPD analysis for detection and fingerprinting of sunflower mutants induced by sodium azide. Int. J. Plant Breed. Genet., 5: 277-285.

Mostafa, G.G., 2011. Effect of sodium azide on the growth and variability induction in *Helianthus annuus* L. Int. J. Plant Breed. Genet., 5: 76-85.

NFHS 3, 2006a. National Family Health Survey. Ministry of Health and Family Welfare, India.

NFHS 3, 2006b. Nutrition and Anemia. International Institute of Population Science, India, pp: 271-274.

Nakagawa, N., T. Anai, A. Okabe, K. Takahashi, M. Hajika and T. Takagi, 2011. Mutation Breeding of Soybean in Japan. In: Breeding of Pulse Crops, Kozgar, M.I. and S. Khan (Eds.). Kalyani Publishers, Ludhiana, India, pp: 55-84.

Novak, F.J. and H. Brunner, 1992. Plant breeding: Induced mutation technology for crop improvement. IAEA Bull., 4: 25-32.

Parry, M.A., P.J. Madgwick, C. Bayon, K. Tearall and A.H. Lopez *et al*., 2009. Mutation discovery for crop improvement. J. Exp. Bot., 60: 2817-2825.

Singh, M.P., 2010. Spread of Hunger. Frontline, 27: 12-23.

Singh, R.K. and B.D. Chaudhry, 1985. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers New Delhi, India.

Srivastava, P.S.M., P. Pandey and D.K. Tiwari, 2011. Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (*Triticum aestivum* L. em. Thell.). Asian J. Plant Sci., 10: 190-201.

Tah, P.R., 2006. Induced macromutations in mungbean [*Vigna radiata* (L.) Wilczek]. Int. J. Bot., 2: 219-228.

Toker, C., S.S. Yadav and I.S. Solanki, 2007. Mutation Breeding. In: Lentil: An Ancient Crop for Modern Times, Yadav, S.S., D. McNeil and P.C. Stevenson (Eds.). Springer, Dordrecht, pp: 209-224.

Wani, M.R. and S. Khan, 2006. Estimation of genetic variability in mutated population and scope of selection for yield attributes in *Vigna radiata* (L.). Wilczek. Egypt. J. Biol., 8: 1-6.

Yaqoob, M. and A. Rashid, 2001. Induced mutation studies in some mungbean (*Vigna radiata* L.) Wilczek cultivars. J. Biol. Sci., 1: 805-808.

Zhang, J., D. Guo, Y. Chang, C. You and X. Li *et al.*, 2007. Non-random distribution of T-DNA insertions at various levels of the genome hierarchy as revealed by analyzing 13 804 T-DNA flanking sequences from an enhancer-trap mutant library. Plant J., 49: 947-959.