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GENETIC IMPROVEMENT OF FOXTAIL MILLET FROM KOLLI HILLS LANDRACES THROUGH CHEMICAL MUTATION

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ABSTRACT Human beings and their culture evolved along with their development in agricultural practices. In which variety food crops were identified, breed and improved through natural selection and their performance contributing to their huge agro biodiversity. Foxtail millet, S. italica is one which was attempted in the present study to improve through chemical mutation. In the present study *S. italica* seeds were collected from Kolli Hills, Tamilnadu, India and exposed to different concentration of chemical mutagen Ethyl Methanesulfonate (EMS) and found that percentage germination, root length, shoot length, seedling length Height, and vigour index of *S. italica* reduced with increasing concentration of EMS. However, days of flowering of *S. italica,* decreased and increased, productive tillers, panicle length, average 100 grain weight, proline content, Melanodialdehyde (MDA), H2O2, ascorbic acid, DPPH activity, protein, fibre, energy, riboflavin and folic acid of S. italica increased and decreased with increasing concentration of EMS. Productive tillers, panicle length, Melanodialdehyde (MDA), H_2O_2, ascorbic acid, DPPH activity, protein, fibre, energy and folic acid of S. italica were high at 0.45% EMS. The average 100 grain weight and riboflavin of *S. italica* was higher at 0.30% EMS. MDA of S. italica was high at 0.60% of EMS and proline content of *S. italica* increased all along the increasing concentration of EMS.

KEYWORDS : Millets, EMS, nutrient

Introduction

Millets are the traditional food crop in peninsular Indian sub continent. These millets are annual grass species which provided nutritious food and fodder mostly Paniceae which include 15,000 species widely cultivated in tropical and subtropical regions of the world (de Wet, 1987; Soreng *et al*., 2015). The archaeological findings on agricultural crops millets formed one of the important crops. Among the 84 genera Pannicum, Setaria, Echinochola, Pennisetum, Paspalum and Eleusine are widely cultivated in the India and Asian subcontinent which are referred as small millets (Dendy, 1995). Millets vary in their morphology, plant height, inflorescence structure, flowering period, maturity period, grain colour and shape, etc., (Reddy *et al.*, 2006). However, India is the major producer of these crops has been 43.85% production of world (Chandel *et al.*, 2014). Among the different millets Setaria italica which is commonly known as foxtail millet is the second largest millet cultivated and consumed in Asia apart from Finger millet. Around 1535 *S. italica* accessions were reported to be collected from 26 countries by International Crops Research Institute (ICRISAT) in India (Pinghua Li and. Brutnell, 2011). Most of the S. italica landraces are excellent drought tolerant crop suitable to arid conditions apart from being nutritionally rich, digest slowly and provide energy throughout the day (Gopinath, 2004). Though numbers of attempts are being made for the past few decades still needs extensive research in evolving high yielding varieties of *S. italica*. Hybridization through breeding and selection is an effective method in evolving elite varieties it is time taking process which may not ensure all favourable agronomic and quality characteristics. Though hybridization through recombinant breeding for genetic improvement small inflorescence of *S. italica* is the limiting factor hence, it has been identified that mutation breeding has advantage over time and agronomic and quality characteristics. Mutation leads to sudden heritable genetic alteration in an organism without genetic segregation and recombinant process. Such mutations could be induced through chemical physical and biological agents (Roychowdhury and Tah, 2013). Millets posses multiple alleles which are the potential source of genetic diversity and mutation improvement of the crop (Oladosu *et al*., 2016). Variety of mutation studies were carried by different researchers like divergence of finger millet was evolved by Muduli and Mishra (2007), dwarf varieties by Uma and Salimath (2001), Senapati and Misra (2009) produced high yielding varieties in blackgram through physical and chemical mutagens. In the light of this the present research attempts to improve the S. italica with different concentration of chemical mutagen.

Methodology

The foxtail millet (Setaria italica) was collected from Kolli hills Namakkal, Tamilnadu, India. Seeds were surface sterilized using 2.5% sodium hypochloride and germinated in the petriplates with moisture content for three days. Germinated seedlings were transferred to hydrophonic system and MS media was supplemented at regular intervals as described earlier (Verslues et al., 1988).

Setaria italica seeds were treated with the EMS in five different concentrations (0.15%, 0.30%, 0.45%, 0.60% and 0.75%) along with control. One hundred seeds were used for each treatment. Seed was presoaked in water for 10 hours and soaked with EMS for 6 hrs. The observation on germination and seedling growth were taken on 9th day after sowing by counting the number of seeds germinated for each treatment and recorded the germination, root, shoot, seedling length vigour index and from the germinated seedlings.

After the treatment the grown millets was harvested and the observation about the plants such as Days to flowering, height of the plant(cm), productive tillers per plant, panicle length (cm) and 100 grain weight (gm) was measured.The grown millets was determined for Proline (Bates *et al.*, 1973), malondialdehyde (MDA) (Hodges et al., 1999), Ascorbic Acid (Omaye *et al.*, 1979), Hydrogen Peroxide (Roychoudhury et al., 2007), DPPH –free radical, (Kumaran and Karunakaran, 2006), Protein (Bradford, 1976), Fat Content (AOAC: Official methods of analysis, 1965) and Riboflavin by Fluorometric Method,

Results

The germination percentage was significant with all the concentration with p value<0.0001 and f value 208.64, root length(cm) was significant with all the concentration with p value<0.0001 and f value 152.01, Shoot length(cm) was significant with all the concentration with p value<0.0001 and f value 302.62, seedlings length(cm) was significant with all the concentration with p value<0.0001 and f value 116.96 and vigour index was significant with all the concentration with p value<0.0001 and f value 343.63 (Table 1).

The production and productivity of *S. italica* under different EMS treatment showed that days to flower the EMS treatment at 0.75% showed high and all the concentration showed significant with p value <0.0001 and f value 73.82, height of the plant was high in control and

all the concentration showed significant with p value <0.0001 and f value 87.775, productive tillers per plant showed high in 0.45% treated EMS and 0.45% concentration of EMS only showed significant others are non significant with p value 0.0037 and f value 6.556, panicle length of the plant was high in 0.45% treated EMS, 100 grain weight was high in 0.30% treated EMS (Table 2).

Biochemical production of *S. italica* under different EMS treatment showed that high concentration of proline was observed in 0.75% treated EMS, all the concentrations showed significant p value <0.0001 and f value 2177.8, MDA was observed high in 0.45% treated EMS, all the concentrations are significant with p value <0.0001 and f value 123.56, H_2O_2 was observed high in 0.45% treated EMS, all the concentrations are significant with p value <0.0001 and f value 123.56, Ascorbic acid was observed high in 0.45% treated EMS, all the concentrations are significant with p value <0.0001 and f value 605.60, and DPPH was observed high in 0.45% treated EMS, all the concentrations are significant with p value <0.0001 and f value 243.73 (Table 3).

The nutrient status of S. italica under different concentration of EMS treatment were as follows protein, fibre, Folic acid, energy content showed high in 0.45% treated EMS and the riboflavin content was high in 0.30% treated EMS (Table 4).

Discussion

Industrialization process lead to exponential growth of human population and natural resource utilization for his comfort living. In the context human beings are compelled to increase production and productivity of agriculture produce, which lead to evolution of high yielding varieties which increased several times of its productivity per unit area. However, this leads to concentration few major crops which lead to reduction in diversity of food basket, homogenous food habit and mono culture of crops. These conditions further disturbed the ecology and balance in the human diet. Millets provided not only provided food it also provided feed and maintained the productivity of soil ecology. The improvement in the agronomy of few crops reduced the height, duration of flowering and seed production is the important factor which lead to their wide spread cultivation and consumption. I may not be logic in the present context to encourage farmers to cultivate millets and compete with such high yielding varieties. Hence, high yielding varieties of millets are important for their cultivation and human nutritional security. Mutation could be carried out through physical and chemical means, physical means through high energy light source which may further revert in due course. Mutation through chemical means could alter the transposons which may have permanent or long term stability. In the present study different concentration of Ethyl methanesulfonate (EMS) was used as chemical mutagen for S. italica seeds. The seed germination, root length, shoot length seedlings length reduced significantly with increasing concentration of EMS compared to control. The decrease in seed germination was also recorded in millets and other crops by many researchers (Kumar and Mishra, 2004; Eswari, *et al*., 2014). The days of flowering significantly decreased up to 47.33±0.58 with concentration up to 0.45 % of EMS and increased with further increase in concentration. Eswari *et al*., (2014) showed that EMS treatment decreased the days of flowering and decreases with increase of EMS concentration in finger millet and linseed respectively. However, Girhe and Choudhary (2002). Height of the plant decreased with increasing concentration of EMS in the present study which was also observed by Eswari *et al*., 2014 in finger millet. Number of productive tillers and panicle length increased with treatment of EMS and high with 0.45% EMS in the present study but Eswari *et al.*, (2014) and Muduli and Mishra (2008). showed that productive tillers decreased with increasing EMS treatment. Average 100 gram seed weight initially increased with increase in EMS treatment in the present study with *S. italica* and reduced from 0.30% EMS treatment, the weight of seeds was significantly higher at 0.30% EMS. The proline concentration of S. italica increased with increased concentration of EMS proved their stress due to the treatment. However the MDA increased up to 0.60% and decreased further, H2O2, ascorbic acid and DPPH activity increased up to 0.45% and decreased further indicating that their stress tolerance due to EMS up to 0.45%. Further confirming the above the protein, fibre, energy and riboflavin was also high up to 0.45% of EMS.

Conclusion

Archaeological evidences showed that millets are one such group of agricultural crop which was once cultivated and consumed widely from time immemorial. In the recent past only few crops like rice, wheat and maize received attention and improved their productivity and their consumptions quality, where as millets are neglected and underutilized due their inferiority in productivity and consumption criteria of present generation. However, it is realized that empirically selected millets are best suited not only to agro ecological conditions but also human beings residing in the region for their balanced nutrition. Among the different crop improvement techniques mutation were proved to be effective in sustainable development of crop but their improved is only random process.

Tables Table 1. Germination and growth of *S. italica* under different EMS treatment.

EMS Treatment	Germination %	Root length (cm)	Shoot length (cm)	Seedlings length (cm)	Vigour Index
Control	96.00±1.00	5.72±0.08	4.49±0.10	10.82±0.24	1038.56±12.72
0.15	91.67±0.58**	5.25±0.07**	4.08±0.03**	10.01±0.13**	917.63±17.94**
0.30	89.00±1.00**	5.07±0.04**	3.81±0.06**	8.98±0.26**	799.35±13.80**
0.45	85.33±0.58**	4.89±0.03**	3.54±0.02**	8.32±0.21**	709.92±15.33**
0.60	81.00±1.00**	4.69±0.07**	3.17±0.05**	7.94±0.10**	643.48±16.24**
0.75	77.67±0.58**	4.50±0.06**	3.06±0.02**	7.62±0.21**	591.86±18.62**
p value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
f value	208.64	152.01	302.62	116.96	343.63

Table 2. Production and productivity of *S. italica* under different EMS treatment.

EMS Treatment	Days to flower	Height (cm)	Productive tillers per plant	Panicle length (cm)	100 grain weight (gm)
Control	56.00±1.00	84.33±2.08	7.33±0.58	11.2±0.50	0.54±0.02
0.15	52.33±0.58**	76.33±1.53**	7.67±0.58ns	10.27±0.50ns	0.48±0.03**
0.30	50.00±1.00**	71±10**	7.67±0.58ns	10.77±0.38ns	2.48±3.22**
0.45	47.33±0.58**	66.67±1.53**	10±10**	13.63±0.51**	0.68±0.01**
0.60	52.33±0.58**	63.33±1.53**	7.67±0.58ns	10.6±0.53ns	0.54±0.01ns
0.75	57.33±0.58ns	62.33±1.53**	7.67±0.58ns	9.4±0.20**	0.5±0.02ns
***p* value**	**< 0.0001**	**< 0.0001**	**0.0037**	**< 0.0001**	**< 0.0001**
***f* value**	**73.82**	**87.775**	**6.556**	**30.142**	**5643.0**

Table 3. Biochemical production of *S. italica* under different EMS treatment.

EMS Treatment	Proline µmol/g (FW)	MDA µmol/g (FW)	H2O2 µmol/g (FW)	Ascorbic acid mg/g (FW)	DPPH % (FW)
Control	366.29±11.12	0.01±0.0006	4.23±0.11	16.08±0.76	21.46±0.90
0.15	417.55±60**	0.02±0.0006**	5.18±0.06**	25.89±0.64**	25.79±0.83**
0.30	517.63±8.01**	0.02±0.0010**	6.2±0.05**	31.94±0.59**	30.7±0.56**
0.45	755.88±7.44**	0.03±0.0015**	8.31±0.09**	54.76±1.39**	42.31±0.79**
0.60	781.37±5.41**	0.04±0.0015**	7.4±0.49**	44.24±1**	33.33±1.02**
0.75	807.21±3.61**	0.03±0.0010**	6.71±0.23**	32.5±1.12**	27.99±0.57**
***p* value**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
***f* value**	**2177.8**	**274.24**	**123.56**	**605.60**	**243.73**

Table 4. Nutrient status of *S. italica* under different EMS treatment.

EMS Treatment	Protein g/100gm	Fibre g/100gm	Energy K.Cal./100	Riboflavin (mg/100g)	Folic acid (µg/100g)
Control	19.77±0.39	6.07±0.27	247.53±9.1	0.07±0.0067	11.78±0.35
0.15	10.52±0.22^{ns}	7.09±0.20**	278.77±3.70**	0.09±0.0055**	13.35±0.35**
0.30	11.72±0.41**	8.06±0.17**	281.81±5.57**	0.15±0.0035**	13.81±0.07**
0.45	15.85±0.26**	10.54±0.26**	331.91±6.51**	0.09±0.0020**	15.05±0.19**
0.60	12.1±0.71**	9.42±0.23**	282.52±3.95**	0.07±0.0026ns	12.34±0.45ns
0.75	11.55±0.28**	8.26±0.13**	263.56±2.05*	0.07±0.0020ns	11.83±0.04ns
p value	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
f value	**78.583**	**164.35**	**76.364**	**169.16**	**60.745**

Ns-non significant; * -less significant, ** - significant, *** highly significant

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