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Isolation of desirable mutants in safflower for crop improvement

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Abstract

The present investigation was undertaken to isolate mutants having promising traits for safflower (*Carthamus tinctorius* L.) improvement. Two varieties of safflower *viz*., AKS-207 and Bhima were mutagenised with ethyl methanesulphonate, sodium azide and gamma rays. All the mutagens decreased pollen fertility in M_1 generation with the increase in their dose. The screening of M_2 population led to the isolation of several putative mutants. The frequency of mutations varied according to the mutagen, its dose and the variety concerned. The evaluation of these putative mutants in M_3 generation resulted in selection of early bolting, dwarf, highly branched, large head, high test weight, thin hull, high oil content and high oleic acid mutants on the basis of their utility in breeding for safflower improvement. Amongst the three mutagens used, sodium azide proved to be most effective and efficient mutagen for induction of mutations in safflower.

Key words: *Carthamus*, effectiveness, efficiency, oleic acid, mutagens, oilseed

Introduction

Over three-fourth of global vegetable oil production is contributed by soybean, oil palm, rapeseed and sunflower. Due to domination of these four oilseed crops, many others are either underutilized or neglected (Murphy 1999; Khan et al. 2009). However, they posses certain useful features. The safflower (*Carthamus tinctorius* L.), although neglected, is a hardy crop suited for tropical and dry regions like India. Moreover, the crop yields oil which is considered good to taste, cook and health (Singh and Nimbkar 2006). The limitations of this crop are spininess, low seed yield and low oil content (Dajue and Mundel 1996). These limitations make safflower a weak competitor with, rapeseed and soybean, which are spineless, having high seed yield and oil content (Pahalvani 2005).

Safflower cultivation may be advantageous provided improvement is made with respect to drought, high temperature and salinity tolerance, which are a major limiting factors affecting safflower production adversely. Although, overlooked but still several varieties of safflower have been developed world over including India through conventional breeding. However, all these efforts could not boost either seed or oil yield of safflower (Kumar and Srivastava 2010). Analysis of few varieties and breeding lines revealed that increase in seed size or test weight results in thicker hull. A thicker hull, in turn, lowers the oil content in seed. Moreover, negative correlation of seeds per head with heads per plant, test weight and head size have been reported, which also prevent development of high yielding varieties (Ranga Rao et al. 1977; Roopa and Ravikumar 2008; Rampure et al. 2014). The other impediment in development of improved varieties of safflower is the presence of relatively narrow variability in the germplasm atleast, for certain characters. Therefore to enhance genetic variability, exploitation of induced mutagenesis in safflower has been suggested (Rampure et al. 2014). Although mutagens do induce desirable variability in the genome of crops but they also cause deleterious effects. Thus, the choice of mutagen and its dose is made on the basis of its effectiveness and efficiency. A few reports mention about the use of mutagenesis in safflower (Mallikarjunradhaya 1978; Ramchandram and Goud 1983; Velasco et al. 2000; Kotcha et al. 2007) but only the one by Mallikarjunradhaya (1978) mentions about the effectiveness and efficiency of the mutagens in safflower. Hence, a study was planned to isolate

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mutants of safflower having desirable attributes from the mutagenized population of two safflower varieties *viz*., AKS-207 and Bhima.

Materials and methods

Dry, healthy and uniform seeds of vars. AKS-207 and Bhima were procured from Department of Seed Technology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, India. On the basis of previously determined LD_{50} doses 200 seeds of each variety were exposed to 400, 500 and 600Gy of gamma rays at Bhabha Atomic Research Centre, Mumbai using ^{60}Co as radiation source. For chemical mutagen treatment 150 seeds of each variety were treated per concentration of mutagen. The concentrations of ethyl methanesulphonate (EMS) used were 0.2, 0.3 and 0.4% (w/v) for var. AKS-207 and 0.1, 0.2 and 0.3% (w/v) for var. Bhima. In case of sodium azide (SA) 0.005, 0.010 and 0.015% (w/v) concentrations were used for both the varieties. The dry but unirradiated and seeds soaked in distilled water served as control in case of physical and chemical mutagenic treatments, respectively. In case of chemical mutagens, in addition to dry seeds, seeds soaked in distilled water for 3 (3h PSW) and 6h (6h PSW) prior to mutagenization were also treated. Chemical mutagenic treatment was carried out in a shaker at 200 rpm at 25±2°C for 18h. The mutagen treatment was terminated by decanting the mutagen solution and washing the seeds thoroughly with tap water. Lastly, the seeds were soaked in tap water for 2h. The mutagen treated seeds were sown in the field during *rabi* of 2010-11. All the recommended cultural measures like spacing between plants and rows, irrigation, weeding, spraying and plant production methods were carried out during the growth period of the crop.

In the M_1 generation, pollen sterility was used to assess lethality induced by mutagens. It was determined on the basis of acetocarmine stainability, which was observed at flowering stage in randomly selected 10 plants per treatment. Dark stained and normal sized pollen grains were considered as fertile and those of irregular shape with light or no stain were scored as sterile.

Seeds of all harvested M_1 plants were sown on plant-to-row basis to raise M_2 generation in the next season (*rabi*). About 50 plants per row were maintained after thinning. The M_2 population was screened for various morphological, yield and biochemical characters such as days to bolt, days to flower, flower colour, spininess, head size, plant height, number of primary branches per plant, number of heads per plant, days to mature, seed size, hull thickness, test weight, seed yield per plant, oil content and fatty acid profile as described earlier (Rampure et al. 2014). The attributes of M_2 progenies which exceeded the range defined by control plants for a particular character were isolated as putative mutants. Mutagenic effectiveness, efficiency and factor of effectiveness were calculated using the formulae given by Rao and Rao (1983) and Kharkwal (1998).

Mutagenic effectiveness = Mf/tc or Mf/Gy

Mutagenic efficiency = Mf/S

where, Mf = Mutation frequency in M_2 generation, t = Period of treatment in case of chemical mutagen, c = Concentration of mutagen in case of chemical mutagen, Gy = Dose of gamma radiation and S = % pollen sterility.

$$\text{Factor of effectiveness} = \frac{\text{No. of mutations}}{\text{No. of treated seeds}} \times 100$$

Mutation rate, which gives an idea about mutations induced by particular mutagen irrespective of dose was calculated by the following formula (Shirsat et al. 2010; Satpute and Fultambkar 2012).

$$\text{Mutation rate} = \frac{\text{Sum values of effectiveness or efficiency of a particular mutagen}}{\text{No. of treatments of that particular mutagen}}$$

The seeds obtained from these putative mutants were sown next year to raise M_3 population to study their breeding behaviour.

Results

The pollen fertility was decreased by all the mutagenic treatments in the M_1 generation mostly in a dose-dependent manner in both the varieties (Table 1). In M_2 generation several putative mutants of vars. AKS-207 and Bhima were isolated. These mutants were early bolting, late bolting, early flowering, late flowering, dwarf, tall, less branched, highly branched, less heads per plant, more heads per plant, small head, large head, early maturing, late maturing, low yielding, high yielding, small seeds, bold seed, low test weight, high test weight, thin hull, low oil content, high oil content, high oil yielding, low linoleic acid, high linoleic acid,

low polyunsaturate-to-saturate ratio (P/S), high P/S, low oleic desaturation ratio (ODR) and high ODR. Most of these putative mutants were isolated from the mutagenised population of both the varieties (Tables 2 and 3).

The mutation frequency varied according to the mutagen, the character concerned and the variety (Tables 1-3). The mutation frequency induced by EMS was comparatively lower in var. AKS-207 than the var. Bhima. Moreover, while the mutation frequency was decreased due to pre-soaking in var. AKS-207, the same was enhanced in var. Bhima due to pre-soaking (Table 1). The mutation frequency due to SA treatment varied in both the varieties according to the concentration. It was nearly equal with 0.005% SA in both the varieties. However, the higher concentrations of SA had the differential effect. In the var. AKS-207 the higher concentration reduced the mutation frequency. However, in var. Bhima 0.010% SA did not induce any mutations, but the mutation frequency

Table 1. Effect of mutagens on safflower varieties, AKS-207 and Bhima

Treatment	AKS-207					Bhima				
	% pollen sterility	% mutations	Effective-ness	Factor of effective-ness	Efficiency	% pollen sterility	% mutations	Effective-ness	Factor of effective-ness	Efficiency
Ethyl methanesulphonate (%)										
0 PSW										
0.1	-	-	-	-	-	24.09	0.62	0.34	4.67	0.03
0.2	10.50	0.69	0.19	4.00	0.07	11.57	1.02	0.28	4.67	0.09
0.3	19.62	2.78	0.51	16.67	0.14	34.31	1.40	0.26	6.00	0.04
0.4	24.47	0.00	0.00	0.00	0.00	-	-	-	-	-
3h PSW	-	-	-	-	-	-	-	-	-	-
0.1						16.43	3.81	2.12	20.00	0.23
0.2	15.51	0.75	0.21	7.33	0.05	22.90	3.19	0.89	13.33	0.14
0.3	19.35	0.00	0.00	0.00	0.00	24.71	3.42	0.63	10.67	0.14
0.4	29.38	0.00	0.00	0.00	0.00					
6h PSW	-	-	-	-	-	-	-	-	-	-
0.1						30.86	2.09	1.16	12.67	0.07
0.2	19.29	0.12	0.03	0.67	0.01	23.12	1.40	0.39	6.00	0.06
0.3	21.34	0.00	0.00	0.00	0.00	51.62	0.00	0.00	0.00	0.00
0.4	45.30	0.00	0.00	0.00	0.00					
Sodium azide (%)										
0 PSW										
0.005	6.82	1.81	20.07	7.33	0.26	1.56	0.17	1.86	1.33	0.11
0.010	19.83	0.83	4.60	2.67	0.04	24.70	0.00	0.00	0.00	0.00
0.015	16.11	0.88	3.28	1.33	0.05	11.11	3.41	12.63	6.00	0.31
3h PSW										
0.005	11.21	0.22	2.48	2.00	0.02	21.37	0.00	0.00	0.00	0.00
0.010	23.00	3.03	16.85	18.67	0.13	26.52	0.57	3.16	3.33	0.02
0.015	30.13	0.84	3.11	2.00	0.03	30.14	0.54	2.01	1.33	0.02
6h PSW										
0.005	7.43	0.90	10.03	5.33	0.12	16.54	0.00	0.00	0.00	0.00
0.010	24.12	0.10	0.55	0.67	0.00	30.18	0.00	0.00	0.00	0.00
0.015	33.43	0.00	0.00	0.00	0.00	32.05	1.27	4.70	4.00	0.04
Gamma ray (Gy)										
400	27.92	1.75	0.04	8.00	0.06	28.32	1.32	0.03	4.00	0.05
500	37.86	4.08	0.08	18.00	0.11	33.27	0.66	0.01	3.00	0.02
600	56.35	0.00	0.00	0.00	0.00	50.29	0.68	0.01	1.00	0.01

Table 2. Putative mutants isolated from mutagenised population of var. AKS-207 in M_2 generation

Mutants	Mutagen		
	Ethyl methanesulphonate	Sodium azide	Gamma ray
Early bolting	-	3h-0.005% (0.07)	0h-400Gy(0.11); 0h-500Gy (0.11)
Late flowering	0h-0.3% (0.67); 3h-0.2% (0.07)	0h-0.005% (0.49)	0h-500Gy (0.68)
Dwarf	-	3h-0.010% (0.11)	0h-500Gy (0.11)
Tall	0h-0.2% (0.12)	3h-0.010% (0.22); 6h-0.005% (0.11)	-
Less branched	0h-0.3% (0.11)		-
Highly branched	-		0h-500Gy (0.23)
Less heads/plant	0h-0.3% (0.11)	3h-0.010% (0.11)	0h-500Gy (0.11)
More heads/plant	-	-	0h-500Gy (0.23)
Small head	-	-	0h-500Gy (0.11)
Large head	-	-	0h-500Gy (0.23)
Late maturing	0h-0.2% (0.23); 0h-0.3% (0.67); 3h-0.2% (0.14)	0h-0.005% (0.49) -	0h-400Gy (0.11); 0h-500Gy (0.34)
Low yielding	0h-0.3% (0.11)	3h-0.010% (0.22)	0h-400Gy(0.11); 0h-500Gy (0.45)
High yielding	-	3h-0.005% (0.07)	0h-400Gy (0.33)
High test weight	-	3h-0.015% (0.28); 6h-0.005% (0.11)	0h-400Gy (0.11); 0h-500Gy (0.11)
Small seed	0h-0.3% (0.11); 3h-0.2% (0.07)	0h-0.005% (0.16); 3h-0.010% (0.76); 6h-0.005% (0.11)	0h-500Gy (0.11)
Thin hull	0h-0.3% (0.56); 3h-0.2% (0.07); 6h-0.2% (0.12)	0h-0.005% (0.33); 0h-0.010% (0.21); 0h-0.015% (0.44); 3h-0.005% (0.07); 3h-0.010% (0.54); 6h-0.005% (0.23)	0h-400Gy (0.44); 0h-500Gy (0.68)
Low oil content	0h-0.2% (0.12)	0h-0.010% (0.21); 0h-0.015% (0.44); 3h-0.010% (0.11)	0h-500Gy (0.11)
High oil content	-	3h-0.010% (0.33)	-
High oil yielding	-	6h-0.010% (0.10)	0h-400Gy (0.33)
Low linoleic acid	0h-0.3% (0.11)	6h-0.005% (0.11)	
High linoleic acid	0h-0.2% (0.12); 0h-0.3% (0.22); 3h-0.2% (0.07)	0h-0.005% (0.16); 3h-0.010% (0.22); 3h-0.015% (0.28)	0h-400Gy (0.11); 0h-500Gy (0.11)
Low P/S ratio	-	0h-0.010% (0.21)	
High P/S ratio	0h-0.2% (0.12); 3h-0.2% (0.14)	6h-0.005% (0.11)	0h-500Gy (0.11)
Low ODR	0h-0.3% (0.11); 3h-0.2% (0.07)	0h-0.010% (0.21); 3h-0.010% (0.11); 6h-0.005% (0.11)	0h-500Gy (0.11)
High ODR	3h-0.2% (0.14)	0h-0.005% (0.16); 3h-0.010% (0.33); 3h-0.015% (0.28)	0h-400Gy (0.11); 0h-500Gy (0.11)

Note: h = Pre-soaking duration in hours, % = Concentration of mutagen, Gy = Dose of mutagen, values in parenthesis indicate the per cent mutation frequency

was enormously enhanced (3.41%) by the concentration of 0.015% SA (Table 1). Similarly, gamma ray induced mutations with the frequency of 1.75 and 4.08% at the dose of 400 and 500Gy, respectively in var. AKS-207. However, in var. Bhima the mutation frequency ranged between 0.66 and 1.32% with gamma ray (Table 1).

The effectiveness of EMS was more in var. Bhima as compared to var. AKS-207. Similarly, while the effectiveness of EMS decreased due to pre-soaking in var. AKS-207; it was increased in var. Bhima due

Table 3. Putative mutants isolated from mutagenised population of var. Bhima in M_2 generation

Mutants	Mutagen		
	Ethyl methanesulphonate	Sodium azide	Gamma ray
Early bolting	-	6h-0.015% (0.21)	0h-400Gy(0.16); 0h-500Gy(0.11)
Late bolting	3h-0.1% (0.13); 3h-0.3% (0.21)	3h-0.010% (0.11)	-
Early flowering	0h-0.1% (0.09); 3h-0.1% (0.63); 6h-0.1% (0.11)	-	0h-400Gy (0.33)
Late flowering	0h-0.3% (0.16); 3h-0.1% (0.25); 3h-0.2% (0.48); 3h-0.3% (0.21); 6h-0.1% (0.11)	3h-0.010% (0.11)	-
Dwarf	0h-0.2% (0.15); 3h-0.3% (0.21)	-	-
Tall	3h-0.2% (0.80); 6h-0.1% (0.22); 6h-0.2% (0.16)	0h-0.005% (0.08); 0h-0.015% (0.38)	0h-600Gy (0.34)
Less branched	3h-0.1% (0.13)	-	-
Highly branched	0h-0.1% (0.09); 0h-0.3% (0.16); 6h-0.1% (0.11); 6h-0.2% (0.31)	-	-
More heads/plant	0h-0.3% (0.16)	-	-
Small head	0h-0.1% (0.09); 0h-0.3% (0.16); 3h-0.1% (0.25); 3h-0.3% (0.21); 6h-0.1% (0.22)	6h-0.015% (0.21)	-
Large head	-	-	0h-400Gy (0.16)
Early maturing	3h-0.1% (0.38); 6h-0.1% (0.11)	-	-
Late maturing	0h-0.2% (0.15); 0h-0.3% (0.16); 3h-0.1% (0.25); 3h-0.2% (0.48); 3h-0.3% (0.21)	3h-0.010% (0.11); 3h-0.015% (0.27); 6h-0.015% (0.21)	0h-400Gy (0.16)
Low yielding	3h-0.1%(0.25); 6h-0.1% (0.11); 6h-0.2%(0.16)	0h-0.015% (0.38)	-
Low test weight	6h-0.1% (0.11)	0h-0.015% (0.38)	-
High test weight	6h-0.2% (0.16)	3h-0.010% (0.11)	0h-400Gy(0.16); 0h-500Gy(0.11)
Small seed	0h-0.1% (0.18); 0h-0.2% (0.15); 0h-0.3% (0.16); 3h-0.1% (0.38); 3h-0.2% (0.32); 3h-0.3% (0.43); 6h-0.1% (0.44)	0h-0.015% (0.76); 6h-0.015% (0.21)	0h-400Gy (0.16); 0h-500Gy (0.11)
Bold seed	-	-	0h-500Gy(0.11); 0h-600Gy(0.34)
Thin hull	0h-0.1% (0.09); 0h-0.2% (0.15); 0h-0.3% (0.16); 3h-0.1% (0.38); 3h-0.2% (0.32); 3h-0.3%(0.43); 6h-0.1%(0.33); 6h-0.2% (0.31)	6h-0.015% (0.21)	0h-400Gy(0.16); 0h-500Gy(0.11)
Low oil content	0h-0.1% (0.09); 0h-0.3% (0.31); 3h-0.1% (0.25); 3h-0.2% (0.16); 3h-0.3% (0.43); 6h-0.1% (0.22)	0h-0.015% (0.38); 3h-0.010% (0.11); 3h-0.015% (0.27)	0h-500Gy (0.11)
High oil content	0h-0.2% (0.15); 3h-0.3% (0.64)	0h-0.005% (0.08)	-
High oil yielding	0h-0.2% (0.15); 3h-0.3% (0.21); 6h-0.2%(0.16)	-	-
High oleic acid	3h-0.1% (0.13); 3h-0.2% (0.16)	-	-
Low linoleic acid	3h-0.1% (0.13); 3h-0.2% (0.16)	-	-
Low P/S ratio	3h-0.1% (0.13); 3h-0.2% (0.16)	-	-
High P/S ratio	0h-0.2% (0.15); 6h-0.2% (0.16)	0h-0.015% (0.38)	-
Low ODR	3h-0.1% (0.13); 3h-0.2% (0.16)	-	-
High ODR	3h-0.3% (0.21)	0h-0.015% (0.76)	-

Note: h = Pre-soaking duration in hours, % = Concentration of mutagen, Gy = Dose of mutagen, values in parenthesis indicate the per cent mutation frequency

to pre-soaking. In contrast to EMS the effectiveness of SA was more in var. AKS-207 than var. Bhima. Although, pre-soaking decreased the effectiveness of SA in both the varieties, but the decrease was more in var. Bhima as compared to var. AKS-207. On the other hand, effectiveness of gamma ray was comparatively more in var. AKS-207 than var. Bhima (Table 1). When effectiveness of EMS treatment was pooled over varieties maximum effectiveness was found to be of 0.2% EMS treatment to 3h PSW (Fig. 1). However, the effectiveness of SA was found to be maximum in case of dry seed treatment when treatments were pooled over varieties (Fig. 2). Similarly, the analysis of data after pooling treatments over varieties it was found that gamma ray is most effective at the dose of 500Gy (Fig. 3). Further, when mutagen was pooled over dose and varieties, SA treatment to dry seeds was found to be most effective (Fig. 4). Lastly, the effectiveness of EMS, SA and

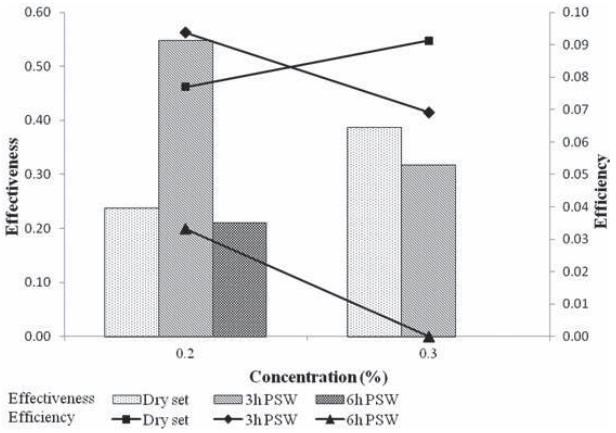

Fig. 1. Effectiveness and efficiency of EMS (treatments pooled over varieties)

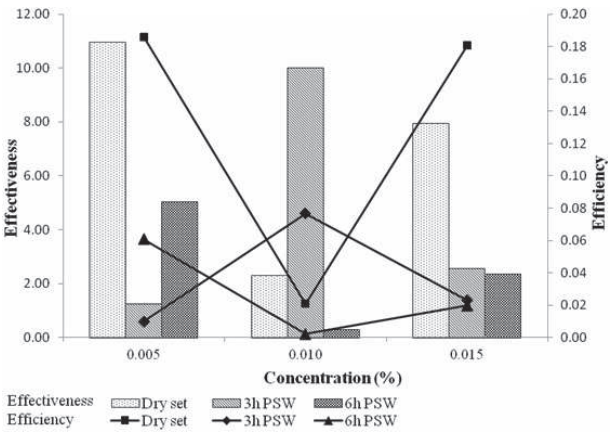

Fig. 2. Effectiveness and efficiency of SA (treatments pooled over varieties)

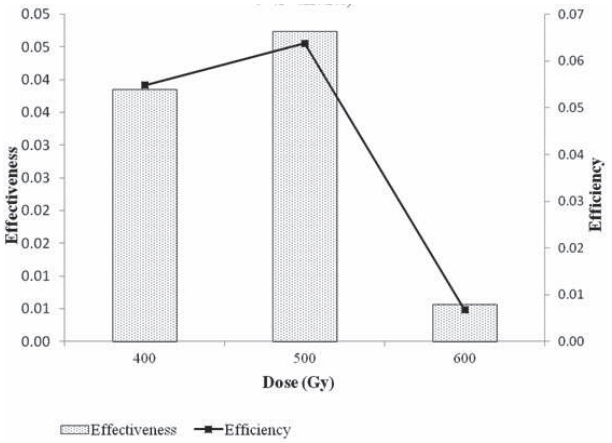

Fig. 3. Effectiveness and efficiency of gamma ray (treatments pooled over varieties)

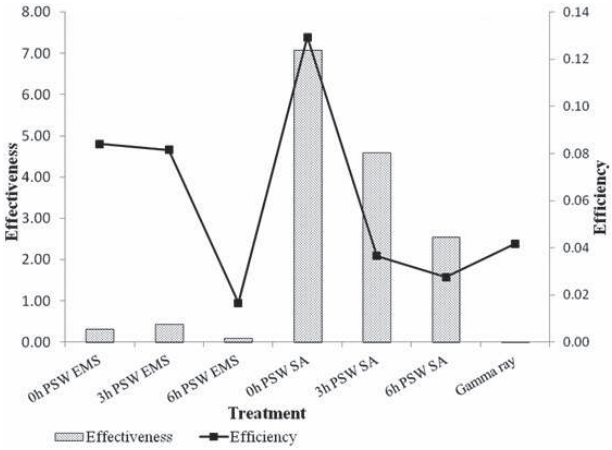

Fig. 4. Effectiveness and efficiency of various treatments (mutagen pooled over concentration/ dose and varieties)

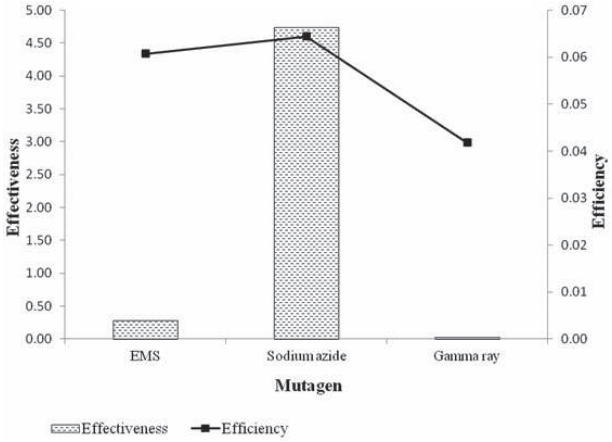

Fig. 5. Effectiveness and efficiency of EMS, SA and gamma ray (mutagen pooled over treatment and varieties)

gamma ray were compared after pooling the mutagen over treatment and varieties. It was found that SA was the most effective mutagen in safflower (Fig. 5).

Like effectiveness, the efficiency of EMS varied with the varieties. It was comparatively lower in var. AKS-207 than var. Bhima. Moreover, the efficiency of EMS decreased due to pre-soaking in var. AKS-207, whereas in var. Bhima the pre-soaking enhanced the efficiency of EMS. In contrast to this, the efficiency of SA was best demonstrated in var. AKS-207 than var. Bhima. However, the soaking of seeds prior to SA treatment reduced its efficiency. Similarly, the gamma ray was found to be more efficient in var. AKS-207 than var. Bhima (Table 1). The pooling of EMS treatments over varieties showed the increase in efficiency of EMS in dry seed treatment with the increase in its concentration. In contrast to this, in pre-soaking treatments, the increase in concentration of EMS decreased the efficiency of EMS (Fig. 1). However, in case of SA the pooling of treatments over varieties revealed minimum efficiency of 0.010% SA treatment to dry and 6h PSW seeds. However, 0.010% SA treatment was found to be most efficient in case of 3h PSW seeds (Fig. 2). The efficiency of gamma ray, when treatment was pooled over varieties, increased from 400Gy to 500Gy. However, with the further increase in the dose to 600Gy the efficiency of gamma ray was decreased (Fig. 3). The pooling of mutagen over dose and varieties revealed that the EMS treatment to 6h PSW seeds decreased the efficiency to a greater extent. Similarly, the soaking of seeds prior to treatment also decreased the efficiency of SA (Fig. 4). A comparison of the efficiency of EMS, SA and gamma ray after pooling of mutagen over treatment and varieties revealed that the chemical mutagen investigated in present study were more or less equally efficient (Fig. 5).

Factor of effectiveness is the measure of the number of mutations per 100 treated seeds. It was 4.00 with 0.2% EMS treatment to AKS-207 seeds and was increased by four times at the concentration of 0.03%. However, the soaking of seeds for 3h prior to treatment with 0.2% EMS increased the factor of effectiveness. In contrast to this the soaking of seeds for 6h prior to 0.2% EMS treatment decreased the factor of effectiveness. On the other hand, the factor of effectiveness of EMS in var. Bhima increased with the soaking of seeds in water prior to EMS treatment. As far as SA is concerned the factor of effectiveness was mostly decreased in both the varieties due to pre-soaking of seeds. The factor of effectiveness in case of gamma ray was mostly higher in var. AKS-207 as compared to var. Bhima (Table 1).

The mutation rate was calculated in the present investigation both in terms of effectiveness and efficiency. The data on two varieties, individually as-well-as on pooling, revealed higher mutation rate in terms of effectiveness than in terms of efficiency. In almost all the cases the mutation rate was maximum with SA as compared to EMS and gamma ray (Table 4).

Table 4. Mutation rate of the mutagen in terms of effectiveness and efficiency

Mutagens	Mutation rate in terms of effectiveness			Mutation rate in terms of efficiency		
	AKS-207	Bhima	Pooled over varieties	AKS-207	Bhima	Pooled over varieties
EMS	0.11	0.67	0.39	0.03	0.09	0.06
Sodium azide	6.77	2.71	4.74	0.07	0.05	0.06
Gamma ray	0.04	0.02	0.03	0.06	0.03	0.04

The seeds putative mutants isolated in M_2-generation were sown in the field to raise M_3 generation. The screening of the M_3 population led to the isolation of a few mutants which can be used in crop improvement programme of safflower. These mutants are described below:

Early bolting: It was isolated in 500Gy gamma ray treatment to var. AKS-207 which bolted in 36 days after sowing, while control bolted in 41-55 days of sowing. This mutant was 125cm tall with 23 branches per plant. There were 90 heads per plant with an average diameter of 22.4mm. The seed yield per plant in early bolting mutant was 49.3g, similar to the parent cultivar. Other characters like, test weight, seed size, hull thickness and oil content were also at par with the parent variety (Fig. 6a).

Plant height: A dwarfmutant was induced by 0.3% EMS 3h PSW treatment in cv. Bhima. The height of this mutant was 74cm as compared to 97 to 130cm in parent. Due to dwarf nature it is resistant to lodging. This mutant was also late bolting, late flowering and late maturing. In addition to this it had lesser branches, smaller head size and lower yield as compared to the parent variety (Fig. 6b).

No. of branches: The highly branched mutant was isolated from the population of var. Bhima treated with 0.1% EMS 0h PSW treatment. The mutant produced 25 branches per plant in contrast to 12 to 20 branches in parent variety. This mutant also produced more number of heads per plant than var. Bhima. The seed yield in this mutant was a little higher than the parent variety, however, the oil content of this mutant was also decreased to 20.7% (Fig. 6c).

Head size: Two large head mutants of var. AKS-207 were induced due to 500Gy gamma ray treatment. These mutants were characterised by the presence of head with the diameter of 31.2 and 33.33mm while the head diameter of parent variety ranged between 20.6 to 23.8mm. The seed yield was also increased in some of the progenies of this mutant (Fig. 6d).

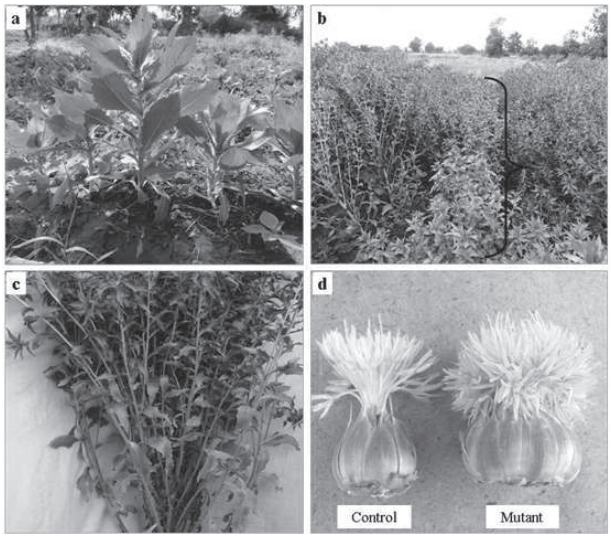

Fig. 6. Mutants of safflower: a: Early bolting, b : Dwarf, c : Highly branched and d : Large head

Test weight: A single high test weight mutant with 8.4g was isolated from 500Gy gamma ray treatment to var. AKS-207, which was about 50% higher than average test weight of parent variety. Similarly, two high test weight mutants of var. Bhima were also isolated from SA and gamma ray treated populations. The test weight in these mutants ranged between 7.1 to 9.4g, which was comparatively higher than the test weight of parent variety (4.2-6.8g).

Hull thickness: A mutant was isolated from 0.010% SA (3h PSW) treated population of var. AKS-207. The mutant had 0.39mm hull thickness as against 0.42 to 0.58mm in the parent variety. The plant height and seed size was reduced in this mutant, while the oil content was increased to 31.7%. The other characters of this mutant were at par with the var. AKS-207.

Oil content: This mutant was induced by 0.010% SA 3h PSW treatment to var. AKS-207. This mutant had 32.7% oil in seed as compared to the 25.4% of var. AKS-207. This mutant also had thin hull, reduced height, less number of branches, small seed size and low yield than the parent variety. The rest of the characters remained unaffected in this mutant.

High oleic acid: The high oleic acid mutants were induced by EMS in var. Bhima as recorded earlier (Rampure et al. 2015).

Discussion

Mutation induction offers the possibility of creating desirable attributes that are either not found in nature or have been lost during the evolution. Thus, selecting a mutagen and its optimum dose for a genotype in any plant species is an important step in mutation breeding programme (Goyal and Khan 2010). It seems that strong mutagen reaches its saturation point at lower doses in the genotypes having highly mutable allele sites. Any increase in the dose of mutagen does not add to the mutation frequency induced by it. It has also been suggested that with the increase in mutagen dose beyond a certain point, the strong mutagens become more toxic than higher doses of relatively weak mutagens (Srinivas and Veerabadhiran 2010). Hence, the present study was focussed to gather the details of the mutagenic effectiveness, efficiency and factor of effectiveness along with mutation rate in two cultivars of safflower.

In general, the effect of mutagen treatment comprises of several parameters. The most important amongst these are dose rate, dose of mutagen, duration of treatment, temperature and pH (Goyal and Khan 2010). The mutagen treatment reduced the pollen fertility in the present study in dose-dependent manner. The similar observations were also recorded by Kumar and Ratnam (2010) in limabean, Kulkarni (2011) and Magar et al. (2012) in soybean. The reduction in pollen fertility has been attributed to the aberrations and some genetic and physiological changes (Kulkarni 2011).

The mutation frequency was grossly decreased at higher doses of a mutagen in the present investigation. However, exceptions to this trend were also noticed. Reports about either increase or decrease in mutation frequency with the increase in dose of

mutagen are available. The increase in dose of mutagen decreases mutation frequency due to increase in pollen sterility and disturbances in formation of enzymes involved in germination process, which decreases per cent germination (Kulkarni 2011). Bolbhat et al. (2012) also reported lower doses of mutagens to exhibit higher frequency of viable mutations. However, exceptionally like in case of EMS and SA treatment to var. Bhima, the mutation frequency was increased at higher doses. These contrasting observations might be due to the fact that exact mechanism and factors which influence mutation frequency are not known. Sometimes, gene mutations without phenotypic expression are usually not recognized (Goyal and Khan 2010). Moreover, the decrease in mutation frequency with the increase in dose shows that a saturation point was reached at higher dose level. Similar observations have been made in SA treatment to lablab (Srinivas and Veerabadhiran 2010). Another factor active in affecting mutation frequency was pre-soaking duration of seeds. Although, a direct connection between mutation frequency and pre-soaking duration has been reported (Badere 2002), but we found an inverse relationship between these two factors. Differences in the mutation frequency and spectrum depend on the interaction of three factors *viz*., mutagen, plant genotype and physiological state of seed at the moment of treatment (Auti and Apparao 2009).

In the present study it was found that effectiveness and efficiency decreased as duration of pre-soaking increased. The pre-soaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in the seeds (Bhosle and Kothekar 2010). Such results have also been reported earlier by Khalatkar (1976). He reported that, there was enhanced EMS uptake in the soaked seeds than in the dry seeds of barley. The soaking duration overlapped the peak of DNA synthesis phase. The soaked seeds, especially in the S_1 phase, have been reported to be more sensitive to mutagen treatments than the dry seeds. The effectiveness of a mutagen also depends on the mechanism of mutagen action. Physical mutagens are highly effective in inducing chromosomal aberrations, whereas chemical mutagens act primarily on base pairs of the DNA molecule and yield a higher number of gene mutations (Van Harten 1998). Due to this mechanistic difference between physical and chemical mutagens; chemical mutagens are generally considered to be superior to physical mutagens for induction of mutations. Girija and Dhanavel (2009) have also reported the results similar to those obtained in the present investigation. They found EMS to be more effective than gamma ray in cowpea.

The present study revealed that the degree of effectiveness and efficiency varied between different mutagens and also between the two varieties. This may be due to the fact that both, effectiveness and efficiency depend not only on the type of mutagen and its dose, but also on the genetic architecture of an organism. The genetic background of material, intracellular condition and perhaps cell cycle also plays an important role in determining the effectiveness and efficiency of the mutagen (Sharma et al. 2008). In the present investigation SA was found to be the most effective mutagen followed by EMS and gamma ray. These findings are in agreement with the reports in linseed (Badere and Choudhary 2007) and clusterbean (Bhosle and Kothekar 2010). However, EMS has been reported to be more effective mutagen in safflower (Mallikarjunradhaya 1978) and blackgram (Bhosle and Hallale 2013). In contrast to these reports, gamma ray has been reported to be more effective in blackgram (Gautam et al. 1992) and soybean (Satpute and Fultambkar 2012).

From breeder's point of view, mutagenic efficiency has more practical value than mutagenic effectiveness (Khan et al. 2005). In this study, SA was found to be the most efficient mutagen amongst those investigated. However, in earlier studies EMS was found to be most efficient mutagen in chickpea (Khan et al. 2005), cowpea (Girija and Dhanavel 2009) and clusterbean (Bhosle and Kothekar 2010). It has also been demonstrated in mungbean that chemical mutagens were more efficient than the physical mutagen (Auti and Apparao 2009). However, reports about the better efficiency of gamma ray than the chemical mutagens are also available (Kumar et al. 2003; Badere and Choudhary 2007).

Along with effectiveness and efficiency, factor of effectiveness and mutation rate were also determined in the present investigation. The factor of effectiveness would be higher if the number of surviving M_1 plants and their fertility was optimal i.e. a certain balance between the surviving M_1 plants and their fertility is necessary (Jagtap and Das 1976). In the present findings highest factor of effectiveness for EMS, SA and gamma ray was 20.00, 18.67 and 18.00, respectively. The factor of effectiveness in rice was high with gamma ray as compared to the chemical

mutagens (Rao and Rao 1983).

The desirable mutagen is the one which is effective as well as efficient for a particular crop (Satpute and Fultambkar 2012). Generally, mutagen dose that gives a highest rate of mutation also induces a high degree of lethality, sterility and other undesirable effects. On the basis of effectiveness, it was observed that mutation rates were high in all the mutagens. However, in terms of efficiency they were low. In soybean the mutation rate with respect to effectiveness was more with EMS than gamma rays. However, gamma ray was found to be the most efficient mutagen (Satpute and Fultambkar 2012).

Thus, due to its higher effectiveness and efficiency in safflower, SA can be the mutagen of choice for safflower improvement through induced mutagenesis. Further, the mutants isolated in present study can be useful for the improvement of safflower varieties which can suitably grown in India. Notably, the high oleic acid mutant is noteworthy, which probably has not been reported in the Indian germplasm.

Authors' contribution

Conceptualization of research (ADC, SJJ, RSB); Designing of the experiments (NHR, ADC, SJJ, RSB); Contribution of experimental materials (NHR, ADC, SJJ, RSB); Execution of field/lab experiments and data collection (NHR); Analysis of data and interpretation (NHR, ADC, SJJ, RSB); Preparation of manuscript (NHR, RSB).

Declaration

The authors declare no conflict of interest.

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References

Auti S. G. and Apparao B. J. 2009. Induced mutagenesis in Mungbean (*Vigna radiata* (L.) Wilczek). *In*: Induced Plant Mutations in the Genomics Era. (ed. Q. Y. Shu) Food and Agriculture Organization of the United Nations, Rome, Italy: 97-100.

Badere R. S. 2002. Investigations on induced mutagenesis in var. RLC-6 and NL-97 of *Linum usitatissimum* (L.) for qualitative and quantitative improvement. Ph. D. thesis. Nagpur University, Nagpur.

Badere R. S. and Choudhary A. D. 2007. Effectivity and efficiency of gamma rays, sodium azide and ethyl methanesulphonate in Linseed. Bioinfolet, **4**(3): 181-187.

Bhosale U. P. and Hallale B. V. 2013. Mutagenic effectiveness and efficiency of gamma rays and ethyl methanesulphonate in black gram (*Vigna mungo* (L.) Hepper). Bionano Frontier, **6**(2): 271-273.

Bhosle S. S. and Kothekar V. S. 2010. Mutagenic efficiency and effectiveness in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). J. Phytology, **2**(6): 21-27.

Bolbhat S. N., Gawade B. B., Bhoge V. D., Wadavkar D. S., Shendage V. S. and Dhumal K. N. 2012. Effect of mutagens on frequency and spectrum of viable mutations in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). IOSR J. Agric. Vet. Sci., **1**(1): 45-55.

Dajue L. and Mundel H. 1996. Safflower *Carthamus tinctorius* L. IPGRI, Rome, Italy and IPK, Gatersleben, Germany.

Elfadl E., Reinbrecht C. and Claupein W. 2010. Evaluation of phenotypic variation in a worldwide germplasm collection of safflower (*Carthamus tinctorius* L.) grown under organic farming conditions in Germany. Genet. Resour. Crop Evol., **57**: 155-170.

Gautam A. S., Sood K. C. and Richarria A. K. 1992. Mutagenic effectiveness and efficiency of gamma-rays, ethyl methanesulphonate and their synergistic effects in black gram (*Vigna mungo* L.). Cytologia, **57**: 85-89.

Girija M. and Dhanavel D. 2009. Mutagenic effectiveness and efficiency of gamma rays ethyl methane sulphonate and their combined treatments in cowpea (*Vigna unguiculata* L. Walp). Global J. Mol. Sci., **4**(2): 68-75.

Goyal S. and Khan S. 2010. Induced mutagenesis in urdbean (*Vigna mungo* L. Hepper): A review. Inter. J. Bot., **6**(3): 194-206.

Jagtap J. G. and Das K. 1976. Mutagenic efficiency and effectiveness of some alkylating agents. Indian J. Genet., **36**(2): 259-26.

Khalatkar A. S. 1976. Influence of DMSO on the mutagenicity of EMS in barley. Botanical Gazette, **137**(4): 348-350.

Khan S., Qurainy F. A. and Anwar F. 2009. Sodium azide: a chemical mutagen for enhancement of agronomic traits of crop plants. Env. Inter. J. Sci. & Tech., **4**: 1-21.

Khan S., Wani M. R., Bhat M. and Parveen K. 2005. Induced chlorophyll mutations in chickpea (*Cicer arietinum* L.). Inter. J. Agric. Biol., **7**(5): 764-767.

Kharkwal M. C., Pandey R. N. and Pawar S. E. 2004. Mutation Breeding for Crop Improvement. *In*: Plant Breeding– Mendelian to Molecular Approaches (ed. H. K. Jain and M. C. Kharkwal). Narosa Publishing House, New Delhi: 601-645.

Kharkwal, M. C. 1998. Induced mutations in chickpea (*Cicer arietinum* L.) frequency and spectrum of chlorophyll mutations. Indian J. Genet., **58**: 465-474.

Kotcha A., Wongyai W., Wongpiyasatid A. And Pongtongkam P. 2007. Gamma radiation induced genetic variability in M_2 population of safflower. *In*: Proceedings of the 45th Kasetsart University Annual conference. Bangkok, Thailand.

Kumar D. S., Nepolean T. and Gopalan A. 2003. Effectiveness and efficiency of the mutagens gamma rays and ethyl methane sulphonate on limabean (*Phaseolus lunatus* L.). Indian J. Agric. Res., **37**(2): 115-119.

Kumar G. and Srivastava P. 2010. Comparative radiocytological effect of gamma rays and laser rays on safflower. Rom. J. Biol. – Plant Biol., **55**(2): 105-111.

Kumar P. R. R. and Ratnam S. V. 2010. Mutagenic effectiveness and efficiency in varieties of sunflower (*Helianthus annuus* L.) by separate and combined treatment with gamma-rays and sodium azide. African Journal of Biotechnology, **9**(39): 6517-6521.

Magar S. P., Mahamune S. E. and Kothekar V. S. 2012. Chemical mutagenesis in soybean (*Glycine max* (L.) Merr). Bioinfolet, **9**(4A): 542-543.

Mahasi M. J., Pathak R. S., Wachira F. N., Riungu T. C. and Kamundia J. W. 2005. Development and evaluation of safflower (*Carthamus tinctorius* L.) cultivars for the marginal rainfall areas of Kenya: Morphological characterization, genetic diversity and adaption studies. Sesame safflower Newsl., **20**: 68-75.

Mallikarjunradhaya K. 1978. Induced mutagenesis in safflower, *Carthamus tinctorius* L. by using gamma rays, ethyl methanesulphonate, alone and in combination. Mysore J. Agric. Sci., **12**(1): 178-179.

Murphy D. J. 1999. The future of new and genetically modified oil crops. *In*: Perspective on new crops and new uses (ed. J. Janick), ASHS Press, Alexandria, VA, USA: 216-219.

Pahlavani M. H. 2005. Some technological and morphological characteristics of safflower (*Carthamus tinctorius* L.) from Iran. Asian J. Plant Sci., **4**(3): 234-237.

Ramchandram M. and Goud J. V. 1983. Mutagenesis in safflower (*Carthamus inctorius* L.).: Differential radiosensitivity. Mysore J. Agric. Sci., **37**(3-4): 309-318.

Rampure N. H., Choudhary A. D., Jambhulkar S. J. and Badere R. S. 2015. Ethyl methanesulphonate-induced high oleic acid mutants in safflower (*Carthamus tinctorius* L.) J. Crop Imp., **29**(6): 720-727. DOI: 10.1080/15427528. 2015.1082950

Rampure N. H., Majumdar P. N. and Badere R. S. 2014. Genetic variability for morphological and biochemical characters in safflower (*Carthamus tinctorius* L.). Indian J. Genet., **74**: 353-361. DOI: 10.5958/0975-6906.2014.00853.0

Ranga Rao V., Ramchandran M. and Arunachalam V. 1977. An analysis of association of components of yield and oil in safflower (*Carthamus tinctorius* L.). Theor. Appl. Genet., **50**: 185-191.

Rao G. M. and Rao V. M. 1983. Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice. Cytologia, **48**: 427-436.

Roopa V. K. and Ravikumar R. L. 2008. Character association studies on cultivars of safflower (*Carthamus tinctorius* L.). Karnataka J. Agric. Sci., **21**(3): 436-437.

Satpute R. A. and Fultambkar R. V. 2012. Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean (*Glycine max* (L.) Merrill). Curr. Bot., **3**(2): 18-20.

Sharma R. K., Gupta B. B. and Bijral J. S. 2008. Effectiveness and efficiency of viable mutations in basmati rice (*Oryza sativa* L.). J. Res., **7**(1): 1-8.

Shirsat R. K., Mohrir M. N., Kare M. A. and Bhuktar A. S. 2010. Induced mutations in horsegram: Mutagenic efficiency and effectiveness. Recent Res. Sci. Tech., **2**(7): 20-23.

Singh V. and Nimbkar N. 2006. Safflower (*Carthamus tinctorius* L.). *In*: Genetic resources, chromosome engineering and crop improvement. (ed. R. J. Singh). Boca Raton, Florida, USA: 167-194.

Srinivas T. R. and Veerabadhiran P. 2010. Efficiency and effectiveness of physical and chemical mutagens and their combination in inducing chlorophyll mutations in M_2 generation of lablab (*Lablab purpureus* (L.) Sweet var. *Typicus*). Electronic J. Plant Breed., **1**(4): 752-757.

Van Harten A. M. 1998. Mutation Breeding- Theory and Practical application. Cambridge Uni. Press, U.K.

Velasco L., Perez-Vich B., Munoz-Ruz J. and Fernandez-Martinez J. 2000. Inheritance of plant height in the dwarf mutant 'Enana' of saffower. Plant Breed., **119**: 525-527.