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Variability Caused by Ethyl Methane Sulphonate in Tomato Fruit and its Nutrient Profile in M1 Generation

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Abstract

Modern techniques of using mutagens to create modifications in the plants for the development of better surviving traits with high yield had gained popularity rather than conventional breeding techniques. Variability in the fruit traits and nutrient profile of tomato fruit was caused by the Ethyl Methane Sulfonate treatments and was done during the year of 2014 in the laboratory of PMAS-AAUR. Water soaked seeds of *Solanum lycopersicum* were treated with EMS of concentrations 8, 16, 24 and 32 mM. Fruits were picked at the breaker stage and observation were made of fruits of the same physiological and horticultural maturity for weight, length, firmness, total soluble solids, vitamin C, total sugars, lycopene, β- carotenes and radical scavenging activity. The results were significant at 5% level. Results revealed that EMS 8 and 16 mM concentrations showed better results as compared to other EMS concentrations. EMS 8 mM concentration was found better in comparison to control and EMS16 mM for fruit weight (72.25 g), fruit length (5.825), TSS (4.15°Brix), Vitamin C (9.543 mg/100 ml), β-carotenes (0.2138 mg/100 ml) and radical scavenging activity (87%). Inferior characters of fruits, fruit firmness (2.36 lb), lycopene contents (0.625 mg/100 ml) and total sugars (1.1075%) resulted due to deleterious effects caused in the genes in response to EMS application. Data was analyzed by statistix 8.1 software.

INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to family Solanaceae and cultivated as annual crop although it is perennial for its valuable fruit yield. It holds the position of second important crop among all vegetables (Grant and Owens, 2002). It has been estimated that 4.58 billion hectares of land fall under tomato cultivation with 150.51 billion tones production and Pakistan gave 530,000 tonnes production during the year of 2011-2012 from 52,300 hectares of land.

Tomato "poor's man orange" is constant source of 74.97 mg vitamin A, 2.56 mg vitamin B, 24.66 mg vitamin C (Imran *et al.*, 2012) polyphenols, naringin flavonoids, rutin and tocopherols (Fruscinate*et al.*, 2007).

Mutagens have unique characteristic of causing variation in the genetic makeup of species and this phenomenon has been exploited to create modifications in tomato but little work is done in regard of changes arise in the nutritive profile as a result of mutagen application in M1 generation. Mutagenesis facilitates the induction of one or more genes modification by keeping the similar genetic makeup (Mahandjiev *et al.*, 2001). Ethyl Methane Sulponate is suggested to cause point mutations. When mutagenesis is being done through seed because it causes wide range of allelic variation within a relatively small population (Emmanuel and Levy, 2002). EMS has been found to be advantageous for causing single locus mutation but without causing harmful mutations of related genes. Mahmoud and Twaty (2006) reported that increase in carotenoid production by 85.71% was due to 4 Krad Gamma rays seed treatment. EMS has capability to induce point mutations which will be able to cause variation in tomato sugar and color. Delayed ripening can be achieved by point mutations which resulted into reduced carotenoid synthesis (Gady *et al.*, 2012) these results are in accordance with Wilde *et al.* (2012) reported mutations at β-galactosidase, expansin genes and ethylene receptors which become the cause of delayed ripening. Present study was conducted in the post-harvest laboratory of PMAS-Arid Agriculture University Rawalpindi under the supervision of Prof. Dr. Nadeem A. Abassi during the year 2014 with an objective of analyzing the changes caused in the tomato fruit and its nutritive profile by the EMS seed treatment because little work has been done in this regard.

MATERIALS AND METHODS

Experimental trial was conducted at the vegetable research area of Horticulture department of PMAS-AAUR and post-harvest laboratory during the year of 2014. This area lies in semi –arid zone which is characterized by humid to sub-humid climate. Tomato seeds of cultivar "Rio Grande" were treated with four different levels of chemical mutagen "Ethyl Methane Sulfonate" (8 mM, 16 mM, 24 mM and 32 mM) and kept for 12 hours at room temperature followed by washing with distilled water. After EMS treatment seeds were sown in the germination trays following four replications for each treatment and kept inside the green house. At 2-4 leaves stage seedlings were shifted to lath house for hardening and kept there for a week and only survived seedlings were transplanted into the field of each treatment comprising four replications. Plants came into bloom after 2 weeks of transplantation and bearing was started after one and a half month of transplantation. Fruits were picked at breaker stage from each replication and brought to post-harvest laboratory for chemical analysis and evaluation of fruits on the basis of physical attributes.Fresh fruits were analyzed for fruit weight, length and firmness followed by the extraction of fruit juice which was used to perform following chemical analysis: Total soluble solids (AOAC, 1990), ascorbic acid contents (Hans, 1992), Total sugars (Horwitz, 1960), Lycopene and β-carotene contents (Nayate, M. and Yanasanta, 1992) and Radical scavenging activity (Rath, 2009). Experiment was conducted by following Randomized Complete Block Design (RCBD) and Analysis of Variance was applied. Least significant difference at 5% level was used to evaluate differences among treatments (Steel *etal.*, 1997).

RESULTS AND DISCUSSION

Fruits were picked at breaker stage and brought to postharvest laboratory of PMAS-AAUR for doing analysis and data is presented in the graphical form. Significant variation was recorded for fruit weight and 8 mM EMS concentration produced fruits of 83.483 g which were heaviest of all treatments. A slight variation for fruit weight is recorded at elevated EMS dose of 32 mM as compared to 24 mM because at highest dose of EMS plants produce less number of fruits which lead to the improvement in fruit weight (Figure 1). Fruit weight is a polygenic trait which is influenced by 11 QTLs in tomato (quantitative trait loci) and *fw2.2* is found to be the major fruit weight determining QTL (Alpert and Tanksely, 1996). Fruit weight is a trait which is also governed by the inward and outward movement of water and carbon from the fruit (Prudent *et al.,* 2009) which means it can be affected by both genetic and environmental conditions.

Capsicum annum produced fruits of heavy weight when seeds were irradiated with fast neutrons for 120 minutes (Falusi *et al.,* 2013). Improvement in the tomato fruit weight was observed when its seeds were treated with Gamma rays 4 Krad and 1 mM sodium azide separately and these results are in accordance with the present study (Mahmoud and Tawaty, 2006).

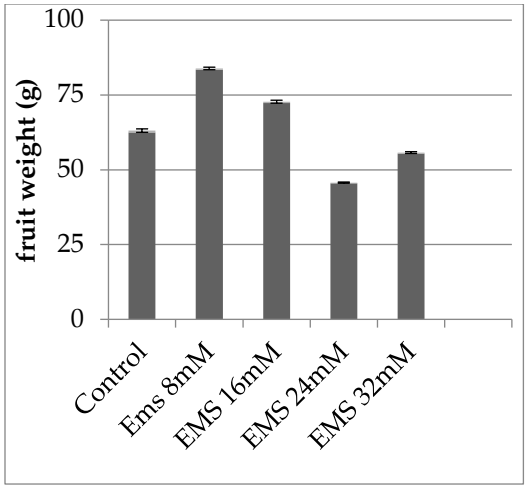

Figure 1: Effect of EMS on tomato fruit weight in M1 generation.

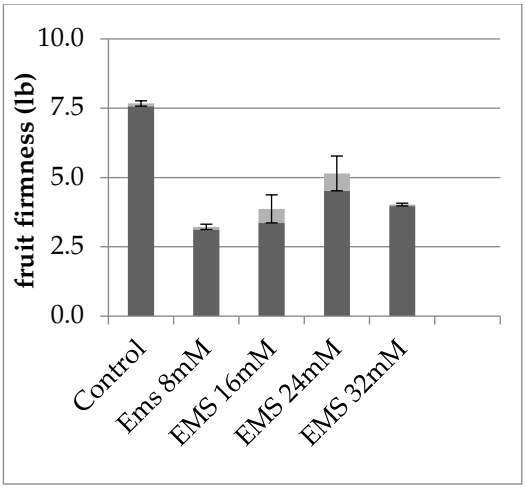

Figure 2: Effect of EMS on Tomato fruit firmness in M1 generation.

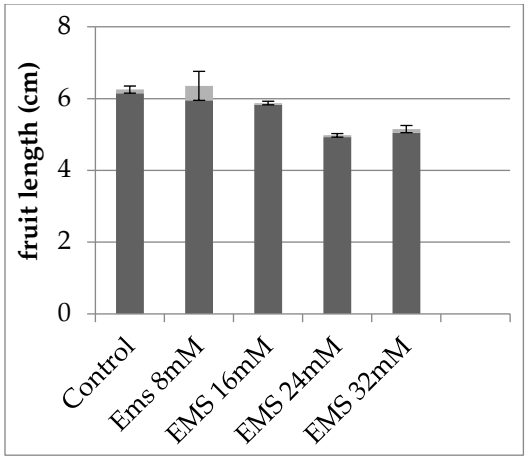

Figure 3: Effect of EMS on Tomato fruit length in M1 generation.

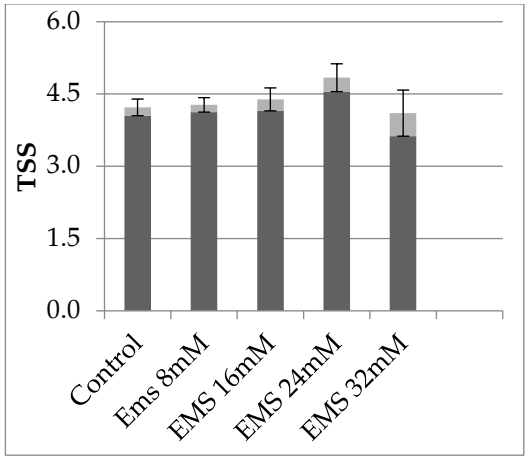

Figure 4: Effect of EMS on TSS of fruit in M1 generation.

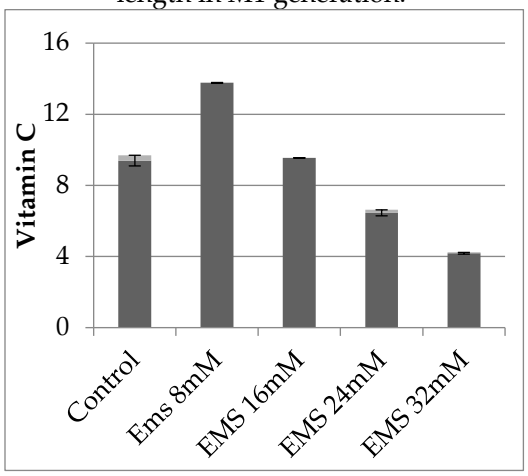

Figure 5: Effect of EMS on vitamin C contents of tomato fruit in M1 generation.

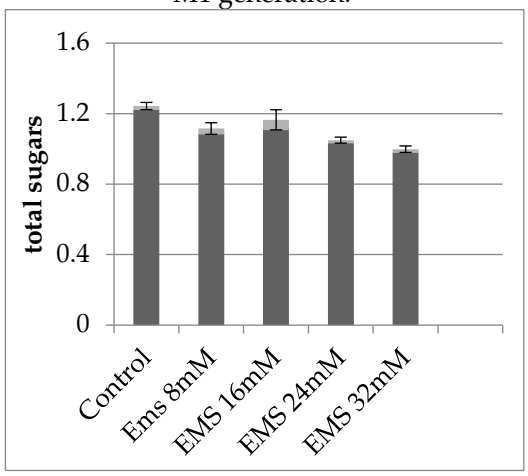

Figure 6: Effect of EMS on total sugars of tomato fruit in M1 generation.

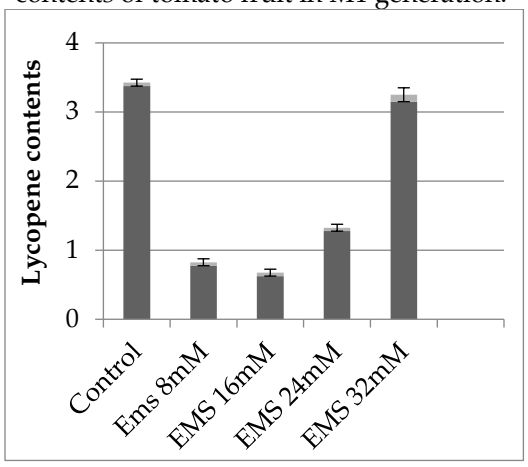

Figure 7: Effect of EMS on lycopene contents of tomato fruit in M1 generation.

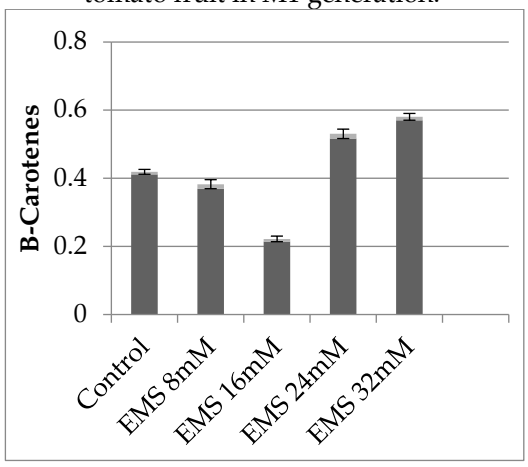

Figure 8: Effect of EMS on β-carotenes of tomato fruit in M1 generation.

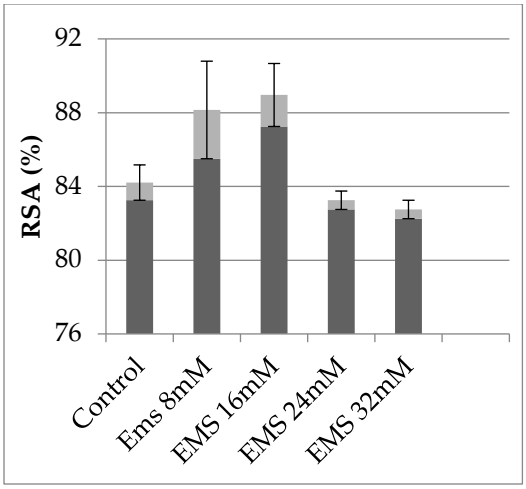

Figure 9: Effect of EMS on RSA of tomato fruit in M1 generation.

Mahmoud and Tawaty (2006) also added into findings that plants with improved chlorophyll contents produce heavy fruits which is according to the presented study like in EMS 8 mM dose (Data not presented). Mutagenic treatments showed negative impact on firmness attribute while non-mutagenic treatment produced firm fruits with firmness of 7.57 lb. Among mutagenic treatments firmness was found to be EMS 8 mM (3.1 lb), EMS 16 mM (2.36 lb), EMS 24 mM (4.5 lb) and EMS 32 mM (3.9 lb) which is lower than non-mutagenic fruits (Figure 2) and this has negative impact on the shelf life of the fruits. Assi *et al.* (1997) reported reduction in fruit firmness after post-harvest irradiation of tomato fruits with Gamma and x-rays. According to Saladie*et al.* (2007) fruit firmness is associated with the presence of strong bonding of hemicelluloses and polysaccharides of middle lamella and polygalacturonase (PG) and expansin proteins work for degradation of cell wall resulting into soft fruits. Alpha expansin gene Le-ESP1 causes loss of firmness in fruits by over expressing itself. Mutagens produce free radicals and cause variation in pairing of nitrogenous bases which become the cause of loss of firmness reported by Jan *et al. (*2011). Mardas*et al.* (2010) developed melon mutants with improved firmness and shelf life through TILLING which is contradiction to the presented study.

Cell wall consists of Glactosyl which induced firmness in fruits by preventing the entry of cell wall destroying enzymes and exo-galactanase work antagonistically to Glactosyl and fruits become soft by facilitating the loosening of polysaccharides and hemicelluloses (Smith *et al.,* 2002). One of the important components of cell wall is pectin and its degradation causes loss of firmness. Matteo *et al.* (2010) found that elevated amounts of ascorbic acid contents cause disintegration of pectin and make fruits less firm. Change in enzymatic activity is due to mutagen application and EMS mutagen these treatments have negative effect on the shelf life of fruits by reducing the fruit firmness (Ehlenfeldt and Jr, 2002).

Fruit length showed significant variation for analysis of variance. EMS treatment 8 mM produced fruits having length of 5.95 cm and at higher doses of EMS 24 mM and 32 mM fruits of 5 cm length were produced (Figure 3). EMS 10 mM and DES 5 mM caused an increase in length of *Capsicum* fruits but at higher doses, a decrease has been reported by Gandi and Devi (2014). According to Falusi *et al.* (2013) seed irradiation of

African wrinkled pepper with fast neutrons also caused an increase in its length and width which are in accordance with the present study.

Yield and yield related parameters increase due the efficiency of phloem tissues involved in the transportation of carbohydrates from site of synthesis to the sink cells but destruction of phloem tissues causes reduction of yield. Damage to the phloem cells occur at higher doses of Gamma rays so this statement gives a base to the use of appropriate number of mutagens depending upon the type of the plant. Appropriate dose of mutagen cause promotes vegetative growth and has positive effects on yield (Preuss and Britt, 2003; Hegazi and Hamideldin, 2010).

Total soluble solids (TSS) is a trait which is controlled by major genes and gives the indication of sugars present in the juice. TSS of tomato shows significant variation (Figure 4) and maximum value was found (4.55°Brix) at 24 mM of EMS concentration and least level of TSS (3.625°Brix) was found at EMS 32 mM. Tomato mutants with TSS variation were produced by Gamma rays and EMS treatments reported by Nunes and Figueria (2009). Tomato mutants produced by EMS in vitro mutagenesis showed TSS ranges of 3.7^0Brix to 5.5°Brix (Shalaby and Banna, 2013). Total soluble solids in fruit is controlled by many genes and phenomenon of dominance and recessive genes lead to the variation (Solimen *et al.,* 2013). According to Shalaby and Banna (2013) TSS is related with size of fruit, fruits obtained from treatments EMS 8 mM and 16 mM is of larger size in comparison of fruits of treatment EMS 24 mM shown highest level of TSS. Studies have shown that disintegration of carbohydrates facilitates the formation of TSS in fruits but if there are more sink cells then competition arises for carbohydrates reserves and present study showed that EMS may be at certain concentrations eliminates sink cells competition and lead to increase TSS (Beckles, 2012). Analysis of variance showed significant variations for sugars percentage is due to variation caused by EMS at genetic level (Beckles, 2012). Although EMS 8 mM and 16 mM did not show variation 1.0825% and 1.1075% respectively but data showed that by increasing the level of EMS sugars perctange was found to be decreasing (Figure 6) as minimum percentage (0.9088%) was found in the fruits that treated with EMS 32 mM.

Highest ascorbic acid contents 13.762 mg was found in the fruits that treated with EMS 8 mM as compared to non-treated fruits (9.3930 mg). Among EMS treatments, peak value of vitamin C was observed at EMS 8 mM (13.762 mg) concentration and by increasing Ethyl Methane Sulphonate dose vitamic C contents were found to be decreasing and minimum concentration (4.1827 mg) was recorded in EMS 32 mM treatment.

EMS treatments showed significant variation (Figure 5) for ascorbic acid contents and maximum AA (13.762 mg/100 ml) is found in the fruits taken from EMS 8 mM treatment. This value is higher than other treatments EMS 16 mM (9.5433 mg/100 ml), EMS 24 mM (6.4543 mg/100 ml) and EMS 32 mM (4.1827 mg/100 ml). AA is an antioxidant that facilitates the cell division and cell expansion (Loannidi *et al.,* 2008) and this statement is supported by the presented results as fruits picked from EMS 16 mM are of larger size comparatively with poor firmness. AA is a multigeneric trait and related to different parameters like firmness. Higher values of Vitamin C cause degradation of cell wall pectin which cause loosening of cell wall (Stevens *et al.,* 2007; Matteo *et al.,* 2010).

Maximum lycopene contents (3.3750 mg) were shown by the fruits taken from non-treated plants followed by EMS 32 mM (3.15 mg). It has been found that by increasing the concentration of EMS lycopene contents decreased (Figure 7) but EMS 32

mM showed variation due to variation caused by EMS. Lycopene accumulation is facilitated by the presence of Phytoenedesatures (PDS) and Pytoene synthase (PSY 1) at fully ripe condition (Ronen *et al.,* 1999). Elevated levels of lycopene are depicting the high levels of PDS and PSY 1 along with the activity of enzymes preventing the conversion of lycopene into β carotenes. On the other hand, lycopene is a sensitive antioxidant which is decreased at high temperature Dumas *et al.* (2003) EMS treatments have shown poor performance because under high temperature lycopene synthesis has been greatly reduced in comparison to non-treated ones.

Treatment EMS 32 mM recorded maximum value of β carotenes (0.5705 mg/100 ml) in the fruits which was significantly high from other EMS treatments and Control fruits. Dense and compact vegetative growth was observed in EMS 16 mM but very low concentration of β-carotenes (Figure 8) (0.2138 mg). At high concentrations of EMS 24 and 32 mM fruits showed better production of β carotenes as compared to fruit picked from control treatment (0.4118 mg). According to Dumas *et al.* (2003) shading of fruits have been found to be a major cause for reduction of β carotenes in fruits and EMS 16 mM had produced excessive vegetative growth that caused shading to fruits. High temperature (35°C) also seized the production of β carotenes. Similarly, plants produced from higher concentrations of EMS exhibit satisfactory results for β carotenes due to scanty vegetative growth but values were not maximum due to residual effect of EMS. According to Ronen *et al.* (1999) tri alkyl amino compounds hinders the activity of lycopene-β-cyclases enzyme that encodes for β Carotene that is the promising reason for low production of β Carotene. High expression of DXS that closely associated with the carotenes synthesis have been reported to be the major factor for High levels of β carotenes production (Lois *et al.*, 2000). Allelic variations have been attributed for this change that arise from Gamma irradiation (Nunes and Figueria, 2009). According to Mahmoud and Tawaty (2006) 4 Krad and 1 mM sodium azide treatments maximize carotenoids synthesis.

Compounds when subjected to oxidation reduction by removal or acceptance of electrons which are called as Radical scavengers or Antioxidant. Energy producing process produce free radicals in the body but these radical at higher concentration cause cancer, cardiovascular and neurological diseases due to oxidative stress (Ebrahimzadeh *et al.,* 2010). Free radicals are produced by DPPH and maximum absorption (517 nm) was recorded and when tomato samples were treated with DPPH color of DPPH change from violet to yellow. Antioxidant produced by DPPH reacts with free radicals to give low value of absorbance (517 nm) as compared to standard DPPH because antioxidant ability inversely proportional to the absorbance (Kulisic *et al.,* 2004). Fruit having ascorbic acid, carotenoids (lycopene and β-carotene), phenolic and flavonoids compounds do scavenging activity as these compounds are reactive oxygen scavengers. Out of five EMS treatments, treatment of 16 mM concentrations produced higher value of RSA (87.250%) followed by EMS 8 mM (85.50%), 24 mM (82.750%) and 32 mM (82.250). RSA are produced in low quantity as compare to 16 mM and 8 mM concentrations and control. Inhibition percentage of control fruits was 83.250% that was quite lower than EMS 8 and 16 mM treatments. Jan *et al.* (2012) has reported oxidative stress elevated when treated with Gamma irradiation it was due to oxygen reactive species. Antioxidants having high level of inhibition showed greater ability to neutralize free radicals (Padmanabhan and Jangle, 2012). Environmental conditions, cultural practices, variety and geographical zone influence radical scavengers (Romero *et al.,* 2007). The most important antioxidants

lycopene and β-carotenes are found in tomato fruit varies remarkably from plant to plant and even fruit to fruit because their production is highly sensitive to environmental conditions, soil type and plant variety (George *et al.,* 2003). Results of EMS 8 and 16 mM are satisfactory for inhibition percentage and can be utilized to produce mutants with increased RSA.

CONCLUSION

Among all the EMS treatments, EMS 8 and 16 mM gives better results, EMS 8 mM concentration produced fruits with superior fruit weight, length, TSS, vitamin C, sugars and RSA but produced less firm fruits having lower amounts of lycopene and β-carotenes as compared to control. EMS 16 mM also produced fruits with same superior traits but produced minimal concentration of lycopene and β-carotenes as compared to EMS 8 mM. At higher concentrations of EMS fruits showed an improvement in only one nutritive trait like EMS 24 mM produced fruits with maximum TSS and EMS 32 mM gave fruits with highest β- carotene contents. These changes occur due to the modification caused by EMS at gene level along with destruction of many genes leading to inferior nutritive traits. Non-treated fruits remained best for physical traits of tomato fruit (fruit firmness and length) and nutritive analysis (total sugars and lycopene contents) of tomato fruit. Seeds obtained from EMS 8 and 16 mM treatments can be utilized to produce mutants to best traits in M_1 to be remained prevalent in M_2 generation as well.

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