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Morpho-agronomic analysis of three roselle (*Hibiscus sabdariffa* L.) mutants in tropical Malaysia

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Abstract

Conventional hybridization in roselle (*Hibiscus sabdariffa* L.) is difficult due to its cleistogamous nature of reproduction. To overcome this limitation, an intensive mutation breeding program using different doses of gamma radiation was conducted. In total, five generation of three mutants were developed from the variety 'Arab'. A number of Morpho-agronomic and physico-chemical characteristics including plant height, canopy diameter, number of branches per plant, number of fruits per branch, number of fruits per plant, weight of fruits per plant, weight of fresh calyx per plant, weight of dry calyx per plant, weight of capsule per plant of these mutants of roselle (*Hibiscus sabdariffa* L.), designated UKMR-1, UKMR-2 and UKMR-3, were evaluated in tropical environment of Malaysia. Significant differences were observed in morpho-agronomic characters in these three mutants. UKMR-2 showed better performance for weight of fruits per plant (2170.7 g) and weight of fresh calyx per plant (1408.4 g); whereas for number of fruits per branch, number of fruits per plant and weight of capsule per plant UKMR-3 showed better performance. The life cycle of mutants was shorter and their lodging resistant was significantly higher compared to their parents and local check.

Keywords: Roselle mutants, Morpho-agronomic traits, Tropical environment.

Introduction

Roselle (*Hibiscus sabdarifa* L.) is an annual or perennial plant belonging to the large family of Malvaceae and is cultivated in tropical and subtropical regions for its stem, fibers, paper pulp or edible calyx, leaves and seeds (Adamson and O'Bryan, 1981; Wilson and Menzel, 1964; Ahmad et al., 1979). Roselle (*Hibiscus sabdariffa* L.) is relatively a new crop in Malaysia. Genus Hibiscus which belongs to Malvaceae has more than 300 known species which are used as ornamental plants. The origin is believed to be from West Africa. It was introduced into Malaysia in early 1990s. Its commercial planting was first promoted by the Department of Agriculture in Terengganu in 1993 and has now spread to other states. Presently, the planted area is quite small approximately 150 ha. It is locally known as "asam kumbang", "asam susur" and, "asam paya". This crop is mainly used to produce pro-health juice, due to its high contents of vitamin C and anthocyanins that are found in the calyxes. The calyxes are also processed into sweet pickle, jelly, jam and tea to some extent. Roselle is a tetraploid (2n=72) species and therefore their segregating populations need longer time for purification compared to to diploid species. Furthermore, roselle has cleistogamous flowers. Thus, crop improvement through conventional hybridization is very difficult to be carried out (Jain, 1979; Vaidya, 2000). To avoid these limitations, we started mutation breeding to generate new source of genetic variability. The study was carried out in collaboration with Malaysian Nuclear Agency in 1999 to produce better improved cultivars. Mutation is the process, which changes the structure of gene or DNA sequence. Mutations are not common in the environment and in most cases it takes a very long time at very low rates to find a suitable mutant (Yap et al., 1990; Young, 1995). Ionizing radiations and chemical mutagens have been the principal agents employed to increase mutation frequencies in plants (Datta, 1995; Chauhan et al., 1992). The radiations or physical agents include X rays, neutrons, gamma rays, ultraviolet, and laser beams while chemical mutagens are ethyl methane sulfonate (EMS), an alkylating agent and colchicine (Donini and Sonnino, 1998; Narasimba and Bhalla, 1998; Sinha and Chowdhury, 1991). In this study gamma ray was used for development of three roselle mutants and then morpho-agronomic traits of mutants (UKMR-1, UKMR-2 and, UKMR-3) were evaluated with their parental line (Arab) and local check (Terengganu).

Breeding program of roselle in Malaysia

Plant breeding is a process of genetic change to improve the genetic content to a more superior genotype.The investigators from the Institute of Higher Education, University of Malaya (UM) initiated the studies and researches on roselle plant in the year of 1993 (Rusmawati 2004, Mohamad et al., 2002). Later, Malaysian Agricultural Research and Development Institute (MARDI) joined UM in these researches. A research program was initiated at UKM in 1999 to increase genetic variation and germplasm accession for breeding programs. To avoid limitations of the conventional breeding, induced mutation approach was started to generate new genetic

variability and was carried out in cooperation with Malaysian Nuclear Agency to produce better improvement.

Results and Discussion

Plant Characteristics

The results of ANOVA are shown in Table 2. The plant height, canopy diameter, number of branches per plant, number of fruits per branch, number of fruits per plant, weight of fruits per plant, weight of fresh calyx per plant, weight of dry calyx per plant and weight of capsule per plant were measured and included in the analysis.

Plant height

Significant differences in plant height was observed among the varieties (significance level $p<0.01$). Results from Tukey's test showed that UKMR-1 and UKMR-2 varieties were significantly different with their parental variety Arab, but there was no significant difference with the check variety Terengganu. The results also indicated that UKMR-3 variety showed no significant difference when compared to Arab variety but showed significant difference with the control variety for this characteristic (Table 2). The means of plant height for these varieties, shown in Table 3, indicates that Arab variety had the highest mean (146.3 cm) among the varieties. UKMR-1, UKMR-2 and UKMR-3 varieties with height of 123.4 cm, 119.3 cm and 128.2 cm, respectively, were higher than control variety which had only at 104.9 cm. In addition, these three mutant varieties were not significantly different with one another based on results from Tukey's test (Table 2). Short plants are often preferred in breeding programs, such as in rice and wheat crops, because short plants can reduce the lodging problems and also better responds to fertilizers (Yap et al., 1990). However, Chang (2006) found that roselle plant with higher main stem are stronger and does not fall easily in production levels compared to short plants. Similar pattern of variability in germplasm evaluation have been earlier reported by Ibrahim and Hussein (2006) and Koorsa (1987). In box plot analysis, the largest range was observed in mutant UKMR 1 and the lowest range was observed in parental variety Arab (Fig 3)

Canopy diameter/coverage

Significant difference was observed in diameter of the canopy among the varieties (significance level $p<0.01$). Results from Tukey's test in Table 2 showed that UKMR-1, UKMR-2 and UKMR-3 varieties were significantly different with their parental and control varieties for this characteristic. Canopy diameter for UKMR-1 variety was significantly different with UKMR-3 variety based on the result of from Tukey's test (Table 2), while, UKMR-2 variety showed no significant difference with UKMR-1 and UKMR-3 varieties. Among the new varieties, UKMR-3 variety had the widest canopy diameter. UKMR-1, UKMR-2 and UKMR-3 varieties with canopy diameter of 99.2 cm, 107.1 cm and 120.6 cm, respectively, showed better performance than Terengganu variety with canopy diameter of only 74.8 cm but showed lower performance than their parental variety with the canopy diameter of 142.1 cm (Table 3). These results are in agreement with the findings of Thirthamallappa and Sherif, (1991). In box plot analysis, the largest range was observed in mutant UKMR 1 and the lowest range was observed in mutant UKMR 3 (Fig 3).

Number of branches per plant

Results from Tukey's test indicated that UKMR-1, UKMR-2 and UKMR-3 varieties were significantly different with their parental and control varieties for this trait (Table 2). The means for number of branches per plant in this study ranged from 6.2 to 10.4. Based on the results, UKMR-2 variety had the most number of branches among the varieties. In addition, UKMR-1 and UKMR-3 varieties had more number of branches in contrast to the Arab and Terengganu varieties. The mutants showed no significant difference for number of branches per plant when compared to each other (Table 2). Mostofa et al., (2002) also observed variation in number of branches/plant in different growing seasons; however the variations were sometimes significant and sometimes non-significant. In box plot analysis, the largest range was observed in parent Arab and the lowest range was observed in mutant UKMR 3 (Fig 3).

Number of fruits per branch

Significant difference was observed among the varieties for number of fruits per branch at $p<0.01$ significance level (Table 2). The results obtained from Tukey's test indicated that UKMR-1 and UKMR-2 varieties were not significantly different with their parental variety but showed a significant difference with the Terengganu variety (Table 4). The means for number of fruits per branch ranged from 9 to 16.8 (Table 3), in which UKMR-3 variety with the highest mean of 16.8 showed the best performance among the varieties. In box plot analysis, the largest range was observed in mutant UKMR1 and the lowest range was observed in local check Terengganu (Fig 3).

Number of fruits per plant

Analysis of variance on number of fruits per plant showed significant difference among the varieties ($p<0.01$). Results from Tukey's test indicated that UKMR-1 variety was not significantly different with its parental variety Arab but it was significantly different with Terengganu variety, whereas UKMR-2 and UKMR-3 varieties are significantly different with both their parental and control varieties (Table 2). Based on the results shown in Table 3, UKMR-1, UKMR-2 and UKMR-3 varieties showed significant difference with each other for this characteristic. UKMR-3 mutant with the highest number of fruits per plant (173) showed the best performance among the varieties. Results of Tukey's test exhibited that mean number of fruits per plant for UKMR-1 and UKMR-2, with 118 and 143 respectively, was higher compare to the Arab and Terengganu varieties with of 99.9 and 56.3, respectively (Table 3). Mutant varieties showed better performance in contrast to their parental variety Arab and local check Terengganu. Aruna et al., (1989) also observed significant variation in number of fruits/plant during their path analysis in roselle. In box plot analysis, the largest range was observed in mutant UKMR3 and the lowest range was observed in mutant UKMR1 (Fig 3).

Table 1. Description of roselle mutants along with their parental line and local check

Characteristics	Terengganu	Arab	UKMR-1	UKMR-2	UKMR-3
Plant height (cm)	124.0	144.3	111.7	108.7	132.2
Canopy diameter (cm)	127.1	123.8	103.8	108.1	123.9
No. of branches per plant	6.4	6.1	8.2	9.5	9.2
No. of fruits per plant	113.7	101.6	134.4	164.2	182.8
Fruit weight per plant (kg)	1.4	1.8	1.7	2.3	1.9
Calyx weight per plant (kg)	0.8	1.2	0.9	1.5	0.9
Capsule weight per plant (kg)	0.6	0.6	0.7	0.8	1.0
Colour of calyx	Red	Dark red	Red	Deep red	Light green
Fruit weight (g)	12.2	17.9	11.3	15.9	10.1

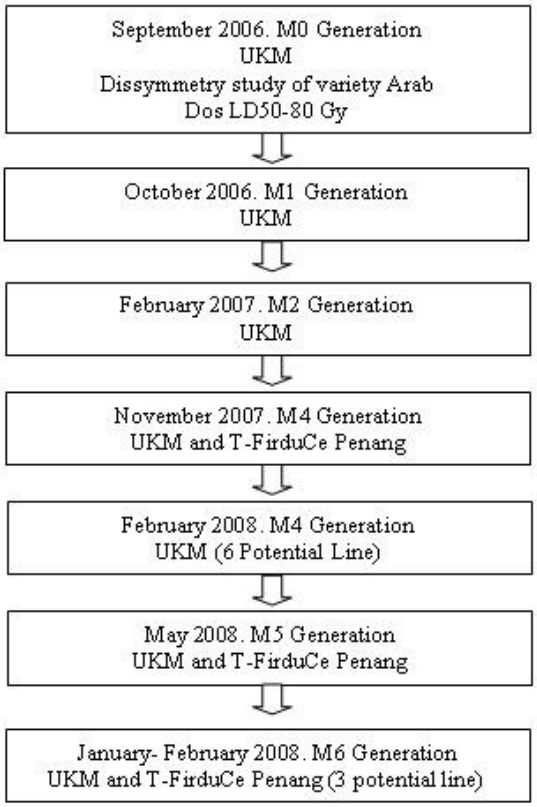

Fig 1. Developmental steps of UKMR 1, UKMR 2 and UKMR 3 roselle mutants

Weight of fruits per plant

Analyses of variance carried out on weight of fruits per plant (Table 2). Based on the results there was a significant difference between varieties for this characteristic at significance level of $p<0.01$. Results from Tukey's test on weight of fruits per plant indicated that UKMR-1 and UKMR-2 varieties were significantly different with their parent and control varieties, whereas UKMR-3 variety showed no significant difference with the parental variety (Table 3). Moreover, UKMR-3 variety showed no significant difference with UKMR-1 variety while it was significantly different with UKMR-2 and Terengganu varieties for this characteristic (Table 2). The means of the weight of fruits per plant shown in Table 3 indicated that UKMR-2 variety with weight of 2170.7 g had highest mean and Terengganu variety with 577.6 g weight of had the lowest mean for this characteristic. These results indicated that the new mutants showed better performance than control variety and UKMR-2 variety is higher in value compare to the parental variety Arab. Significant variations in weights of fruits/plant among the mutants were common and these results are in agreement with the results of Banerjee et al. (1988). In box plot analysis, the largest range was observed in parent Arab and the lowest range was observed in mutant UKMR1 (Fig 3).

Weight of fresh calyx per plant

Based on the results obtained from ANOVA, the varieties were significantly different at significance level $p<0.01$ for weight of fresh calyx per plant (Table 2). Results from Tukey's test shown in Table 3 indicated that UKMR-1 and UKMR-3 varieties were not significantly different from each other but were significantly different with Arab, UKMR-2 and Terengganu varieties for weight of fresh calyx

Table 2. Mean square values from ANOVA of plant height, canopy diameter, number of branches per plant, number of fruits per branch, number of fruits per plant, number of fruits per plant, weight of fruits per plant, weight of fresh calyx per plant and weight of dry calyx per plant

Source of variation	DF	Plant height (cm)	Canopy diameter (cm)	Number of branches per plant	Number of fruits per branch	Number of fruits per plant	Weight of fruits per plant (g)	Weight of fresh calyx / plant (g)	Weight of dry calyx / plant (g)
Varieties	4	2253.2**	6245.3**	30.9**	80.3**	19694.7**	3562486.7**	1772457.9**	7340.9**
Error	45	236.2	240.6	1.45	3.63	365.9	84862.3	41435.6	162.6
Total	49								

**significant at 0.01 levels ($p<0.01$)

Fig 2. Calyx color differences in roselle mutants UKMR 1, UKMR 2 and UKMR 3 along with their parental variety Arab (UKMR1 is red, UKMR 2 is deep red and UKMR 3 is light green and the parent variety Arab's calyx is dark red color)

Table 3. Means of plant height, canopy diameter, number of branches per plant, number of fruits per branch, number of fruits per plant, weight of fruits per plant, weight of fresh calyx per plant, weight of dry calyx per plant and weight of capsule per plant varieties

Varieties	Plant height (cm)	Canopy diameter (cm)	Number of branches per plant	Number of fruits per branch	Number of fruits per plant	Weight of fruits per plant (g)	Weight of fresh calyx / plant (g)	Weight of dry calyx / plant (g)	Weight of capsule per plant (g)
UKMR-1	123.4 bc	99.2c	9.6a	13.1b	118.c	1377.4c	846b	54.7b	529.9b
UKMR-2	119.3bc	107.1bc	10.4a	14.3b	143.1b	2170.7a	1408.4a	83.2a	762.3a
UKMR-3	128.2ab	120.6b	9.9a	16.8a	173.8a	1702.4bc	863.6b	76.4a	838.7a
Arab	146.3a	142.1a	7.7b	12.9b	99.9c	1764.5b	1267.2a	90.5a	497.2b
Terengganu	104.9c	74.8d	6.2b	9.c	56.3d	577.6d	333.5c	23.4c	244c

Means with the same letter within a column are not significantly different at 0.05

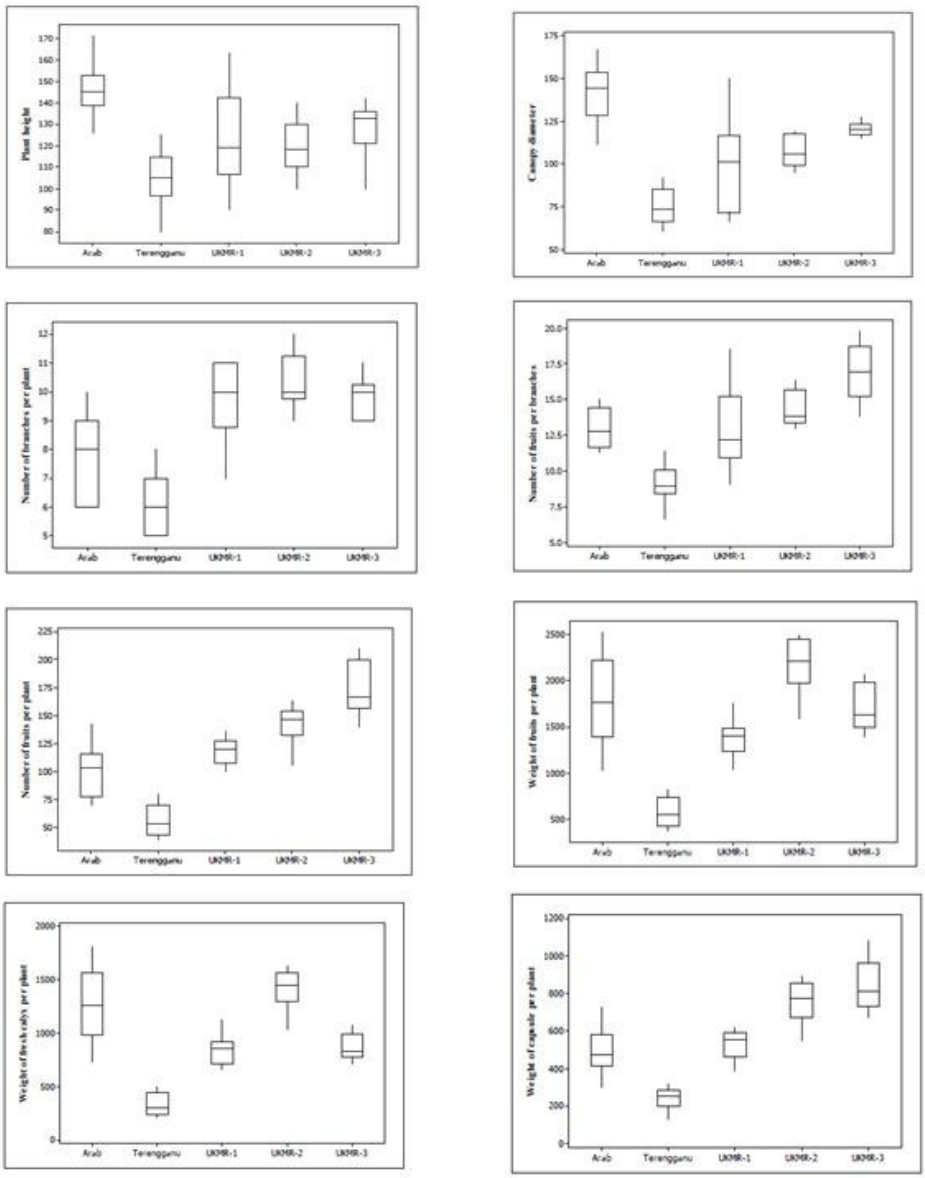

Fig 3. Box plots of morpho-agronomic and Physico chemical traits of three roselle varieties, UKMR-1, UKMR-2 and UKMR-3 compared with two control varieties, Arab and Terengganu

per plant. UKMR-2 variety was also significantly different with Terengganu variety while showed no significant differences with the parental variety Arab for this particular characteristic (Table 2). In this study, fresh calyxes of Terengganu variety had the lowest weight at 333.5 g per plant and, therefore the new varieties showed better performances than the Terengganu variety for this characteristic. UKMR-2 variety with the weight of 1408.45 g per plant showed the best performance when compared to the other varieties. However, KMR-1 and UKMR-3 varieties with the fresh calyx weight of 846.02 g and 863.67 g per plant respectively, showed lower performance than their parental variety Arab, in which the weight was 1267.2 g per plant (Table 3). In box plot analysis, the largest range was observed in parental variety Arab and the lowest range was observed in the local check Terengganu (Fig 3).

Weight of dry calyx per plant

The varieties were significantly different at significance level $p<0.01$ for weight of dry calyx per plant. The UKMR-1 variety showed significant difference with other varieties, while UKMR-2 and UKMR-3 varieties did not show significant difference compared to their parent for the weight of dry calyx per plant (Table 2). Results of Tukey's test also showed that UKMR-2 and UKMR-3 varieties were significantly different with Terengganu variety (Table 3). Terengganu variety had the lowest weight of dry calyx per plant (23.41 g). The average weight of dry calyx per plant for UKMR-1, UKMR-2 and UKMR-3 varieties were 54.75 g, 83.27 g and 76.47 g, respectively, which represent the better performance of new varieties than the control for this characteristic (Table 3). The mean of the weight of dry calyx per plant for Arab variety as shown in Table 3, was 90.50 g

that was the highest average for weight of dry calyx per plant. These results indicate that UKMR-2 and UKMR-3 varieties had higher weight of dry calyx per plant than UKMR-1 variety. The observed pattern of variability in weight of dry calyx/plant were similar pattern of variability have been earlier reported by Aruna et al., (1989) and Banerjee et al., (1988). In box plot analysis largest range was observed in parent UKMR 3 and lowest range was observed in local check Terengganu (Fig 3).

Weight of capsule per plant

Analysis of variance indicated significant difference between varieties at significant level $p<0.01$ for weight of capsule per plant (Table 2). The results from Tukey's test indicated that UKMR-2 and UKMR-3 varieties were significantly different with their parental Arab variety, while UKMR-1 variety showed no significant difference to the parent. These results also showed that these genotypes were significantly different with the Terengganu variety. UKMR-2 and UKMR-3 varieties showed better performance for the weight of capsule per plant compare to the Arab variety with weight of capsule of 497.22 g per plant and Terengganu variety with weight of capsule of 244.03 g per plant. UKMR-3 and UKMR-2 varieties with the weight of capsule of 838.76 g and 762.34 g per plant, respectively, had the highest mean values for this characteristic (Table 3). In box plot analysis largest range was observed in mutant UKMR 3 and lowest range was observed in Local check Terengganu (Fig 3).

Materials and methods

Plant materials

Three mutants namely UKMR 1, UKMR 2 and UKMR 3 were raised in the experimental plot at Universiti Kebangsaan Malaysia along with their parent Arab and local check Terengganu. Brief descriptions of these lines are provided in Table 1 and their appearances are showed in Fig 2.

Methods

Seeds from Arab variety were treated with gamma radiation after dosimetric test (determination of effective gamma irradiation), and were planted at Breeding Experimental Field of National university of Malaysia to detect potential mutants. Dosimetric tests on 2-week-old seedlings showed LD_{50} value of 80 Gy. Later, seeds were treated with gamma radiation from ^{60}Co source using LD_{50} dose to produce M1 generation. Morpho-agronomic data including plant height, canopy diameter, number of branches per plant, number of fruits per branch, number of fruits per plant, weight of fruits per plant, weight of fresh calyx per plant, weight of dry calyx per plant and weight of capsule per plant were taken in due time.

Experimental framework

All of the roselle mutants were grown in Randomized Complete Block Design (RCBD) with two replications. Every experimental unit for each variety framework contains 10 plants (2 reps; 5 plants in each replication) and the total samples of roselle plants that had been planted was 50 (for 5 lines including local check and parental line).

Statistical analysis

MINITAB software (version 14.0) was used to analyze morpho-agronomic data. Analysis of variance (ANOVA) with Tukey's post-hoc comparisons and an alpha level of $p<0.05$ acceptance of the null hypothesis was used to determine significant variables.

Conclusion

Morpho-agronomic traits and physico-chemical characteristics of three new mutant varieties of roselle (*Hibiscus sabdariffa* L.), namely UKMR-1, UKMR-2 and UKMR-3 were evaluated in this study to gather additional attribute of these new mutant varieties developed from Arab variety. Varieties under this study exhibited significant differences for plant characteristics. The new mutants showed better performance in all nine studied parameters compared to Terengganu variety as control variety. UKMR-1, UKMR-2 and UKMR-3 varieties showed better performance than their parent Arab variety for forth parameters of plant characteristics (number of branches per plant, number of fruits per branch, number of fruits per plant and weight of capsule per plant), whereas in plant height and canopy diameter they showed lower performance than Arab variety. UKMR-2 for weight of fruits per plant (2170.7 g) and weight of fresh calyx per plant (1408.4 g) showed the best performance among varieties. UKMR-3 variety for number of fruits per branch (16.8 Numbers), number of fruits per plant (173.8 Numbers) and weight of capsule per plant (838.7 g) showed the best performance between varieties. All the varieties also showed significant differences for fruit characteristics. In general, UKMR-1, UKMR-2 and UKMR-3 mutants performed inferior compared to their parental, Arab variety and showed intermediate performance compared to control line, Terengganu variety for fruit characteristics, with the exception of UKMR-2 for capsule weight per fruit that showed similar performance compare to Arab variety.

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