

## **Mutation breeding for inducing seedlessness in grape variety ARI 516**

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### **ABSTRACT**

The grape variety ARI 516, developed at Agharkar Research Institute, from cross between two grape varieties Catawba and Beauty seedless. It is black coloured, high yielding, early maturing variety having unique musky flavour and good taste with moderate resistance to downy and powdery mildew fungal diseases. Looking into the potential of this variety for table purpose, the mutation breeding program was initiated involving ethyl methyl sulphonate as a chemical and gamma rays as a physical mutagen in different doses. The resulting 175 M<sub>1</sub>V<sub>1</sub> mutant plants including control were planted in the field in 2013. The effect of chemical mutagen on ARI 516 revealed that survival of mutants was more in lower concentrations and shorter duration of mutagenic treatment. High variability was observed in the mutant population. One plant with seedless berries was obtained with 30 Gy gamma irradiation dose in 2017 which was confirmed in later seasons. Six mutants were high yielding and having crunchy bold berries having seed were also obtained with 30 Gy treatment. Another five promising mutants were obtained from 0.1 % EMS treatment for 4 hours and one mutant each for 8 and 12 hours duration respectively. One seeded mutant showed significantly higher performance for yield, the number of berries per bunch, berry length, berry width, 100 berry weight, TSS and trunk girth.

**Keywords:** Gamma irradiation, grape, mutation, seedless mutant

### **INTRODUCTION**

The grape is one of the most important fruit crops grown worldwide. Grapes are considered as rich source of vitamins, minerals and unique natural products like resveratrol. Grapes gaining lot of importance in daily diet due to its anti-oxidant, anti-carcinogenic, immunomodulatory, antidiabetic, antiatherogenic, neuroprotective, anti-obese and anti- aging properties (Yadav *et al.*, 2009). It is grown on an area of 1,39,000 ha mainly in the states of Maharashtra, Karnataka, Tamil Nadu, some parts of North India, Mizoram. About 78 % of grape production in India is utilized for table purpose, 17-20 % for raisin production, 1.5 % for wine and 0.5 % for juice. The seedless varieties are mostly preferred for table purpose in grapes. Presently only a few options like Sharad seedless and its mutants are available in black seedless varieties. More genetically diverse black varieties are needed. MACS-Agharkar Research Institute (MACS-ARI) has developed a hybrid named ARI 516 earlier, which is multipurpose, high yielding variety with unique musky flavour, good taste and moderately resistant to downy and powdery mildew diseases. Being seeded variety, it was gaining less popularity as table purpose. Looking into the potential of this

variety for table purpose, mutation breeding program was started to impart seedlessness and improvement in other characters using chemical and physical mutagens. Among physical mutagens, gamma rays are extensively used in many fruit crops and medicinal plants (Rifnas *et al.*, 2020) for the purpose of creating desire variability among existing population.

### **MATERIALS AND METHODS**

The study was undertaken in MACS-Agharkar Research Institute's farm situated at Hol, Taluka Baramati, District Pune of Maharashtra state. Eight hundred cuttings of variety ARI 516 having 5-6 buds were exposed to both chemical and physical mutagens. Fifty cuttings each were exposed to 10, 20, 30 and 40 Gy Gamma ( $\gamma$ ) irradiation in 2013. The irradiation treatment was given at Bhabha Atomic Research Centre, Mumbai using  $^{60}\text{Co}$  as a mutagenic agent. Mutagen, EMS was used as a chemical mutagen with 0.1, 0.2, 0.3 and 0.4 per cent concentration. Treatment was given to 50 cuttings each for 4 hr, 8 hr and 12 hr duration. The surviving first generation (M<sub>1</sub>V<sub>1</sub>) mutants were planted in nursery bags and then transplanted in the field in due course at spacing 8 x 4 feet. The numbering of mutants was done on the basis of the position on

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the field. The training of mutant plants had been done on the Y trellis. Pruning was done on 8-10th internodes in October. Vines were maintained by applying the recommended dose of fertilizers and other horticultural practices time to time. The data have been recorded for fruiting and disease resistance under natural field conditions. Data on 14 different morphological and phenological characters viz. trunk girth (cm), number of bunches/vine (no.), bunch length(cm), bunch width (cm), peduncle length(cm), yield/vine, number of berries/bunch, bunch weight (g), berry length (cm), berry width (cm), 100 berry weight (g), TSS (0B), number of seeds/berry and time of physiological maturity (days) were recorded during 2017-18 and 2018-19 season. Other qualitative characters like bunch type, bunch density, berry shape, colour and uniformity was also noted during the investigation period. The data were pooled and subjected to statistical analysis for standard deviation(SD), standard error (SE) and coefficient of variance

(CV). The range represented the minimum and maximum values recorded for each trait. The promising mutants were identified on the basis of the performance superior to the mean plus standard deviation for most of the traits studied except the number of seeds/berry. For that trait, less seeds were considered promising.

## RESULTS AND DISCUSSION

Out of 800 cuttings exposed, 307 cuttings were sprouted, and 240 vines which started fruiting were evaluated for the vegetative and fruiting quality. Nineteen cuttings of ARI 516 were planted in the field as control for the evaluation of the comparative performance with mutants. Among the fifty cuttings, each exposed to gamma rays, maximum cuttings (37) were sprouted in 30 Gy treatment, followed by 20 Gy treatment (33), whereas 29 cuttings were sprouted with 10 Gy treatment and only 10 cuttings were sprouted with 40 Gy treatment (Table 1).

**Table 1: Mutation treatments of mutagens on cuttings of ARI-516**

Treatment	Concen- tration	Duration cuttings	No. of cuttings exposed	No. of mutants sprouted	No. of mutants planted in field	Number of (Mutant evaluated	Plant selections Number)
$\gamma$ rays	10 Gy	-	50	29	25	14	-
	20 Gy	-	50	33	25	17	-
	30 Gy	-	50	37	30	26	M-826, M-828, M-831, M-835, M-841, M-870
	40 Gy	-	50	10	10	8	M-886, M-887
EMS	0.1 (%)	4	50	37	25	25	M-947, M-949, M-960, M-974, M-975
		8	50	28	25	19	M-1033
		12	50	27	25	16	M-1098
	0.2 (%)	4	50	18	17	12	M-1159
		8	50	11	11	3	M-1177
		12	50	2	-	-	-
	0.3 (%)	4	50	18	18	10	M-1190
		8	50	2	2	2	-
		12	50	10	7	4	-
	0.4	4	50	-	-	-	-
		8	50	-	-	-	-
		12	50	-	-	-	-
Control	-	-	50	45	20	19	-
Total number of cuttings		850	307	240	175		

Various types of leaf abnormalities like multi-lobed, closed petiolar sinus, deep lobes, prominently serrated and small narrow leaves were observed in mutants. Similar observations were also recorded in earlier reports (Das and Mukherjee, 1968; Sharma and Mukherjee, 1972, 1972a and Sharma and Mukherjee, 1977). Among them, 30 mutants of 30 Gy treatment were planted, and 26 plants were evaluated for morphological and phenological characters when started fruiting in 2017. The number of plants evaluated from 10, 20 and 40 Gy treatment was 14, 17 and 8 respectively. In EMS treatments, maximum sprouting was observed in 0.1 % concentration of EMS, i.e. 37 for 4 hours followed by 8 and 12 hours, i.e. 28 and 27 mutants respectively. The percentage of sprouting and subsequently, survival was observed

diminishing with higher concentration and longer duration of treatment. Cuttings were not sprouted with 0.4 % EMS treatment at all. The survival of mutants was more at lower concentrations and shorter duration of mutagenic treatment. These observations confirm the earlier reports (Kelperis and Daris, 1963 and Das and Mukherjee, 1968; Patil and Patil, 2005). Significant variation was observed among the characters studied when analyzed statistically. Observations for bunch type, bunch density, berry shape, colour and uniformity were recorded which were found similar to the characters of control. All bunches were cylindrical, with black, round berries having uniform maturity. The data on characterization with different characters is presented in table 2.

**Table 2: Statistical analysis of mutants for different characters**

Character	Range	Mean	SD	SE (mean)	CV(%)
Trunk girth (cm)	1.8 - 5.2	3.9	0.52	0.04	13.26
No. of bunches/vine	2 - 68	27.1	10.95	0.83	40.46
Bunch length(cm)	10 - 21	16.8	2.01	0.15	11.93
Bunch width (cm)	3.5 - 7.5	5.6	0.68	0.05	12.08
Peduncle length(cm)	1.8 - 5.5	3.2	0.91	0.07	28.13
Yield/vine kg	0.2 - 7.8	3.2	1.53	0.12	47.40
No. of berries/bunch	23 - 180	117.5	24.71	1.87	21.02
Bunch weight	34.96 - 343.2	185.6	48.73	3.68	26.25
Berry length(mm)	14 - 18	16.6	0.92	0.07	5.57
Berry breadth(mm)	12 - 17	14.8	0.90	0.07	6.06
100 Berry weight(g)	96 - 240	158.1	24.42	1.85	15.44
Time of physiological maturity	121 - 126	123.2	1.39	0.10	1.13
TSS 0 B	13 - 22	17.6	1.72	0.13	9.72
Seeds/berry	0 - 3	1.8	0.54	0.04	29.57

The yield ranged from 0.2 to 7.8 kg/vine. The maximum yield was obtained in M 947 (7.80 kg/plant) followed by M 949 (7.6 kg/plant). Bunch weight ranged from 34.96 to 343.2 g. The Maximum bunch weight was observed in M 902. Hundred berry weight was ranged from 96 to 240 g. The maximum berry weight was recorded in M 826. Physiological maturity ranged from 121-126 days. Early maturity was recorded in M 828, M 870 and M 887. The sweetness of berries was measured in terms of TSS (0B). The maximum TSS was observed in M 1063 (22 0B). All selected mutants showed moderate resistance to downy and powdery mildew.

Six plants of 30 Gy treatment and 2 plants of 40 Gy were selected having significant superiority in important morphological characters over control. Among mutants derived from chemical mutagenesis, 5 mutants of 0.1 % EMS with 4 hrs duration treatment, one plant each with 0.1 % EMS for 8 hrs & 12 hrs., 0.2 % EMS for 4 hrs and 8 hrs and 0.3 % EMS for 4 hrs duration treatment were selected. Total of eighteen promising mutants were selected in the present study having significantly superiority in fruit quality in terms of berry characters and resistance. Performance of selected mutants of ARI 516 for different characters is presented in Table 3.

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**Table 3: Performance of selected mutants of ARI 516 for different characters**

Sr. No.	Mutant No.	Treatment	Trunk girth (cm)	No. of bunches/ vine	Bunch length (cm)	Bunch width (cm)	Peduncle length (cm)	Yield/ vine (kg)	Bunch weight (g)	No. of berries/ bunch	Berry length (mm)	Berry breadth (mm)	Berry weight (g)	Physiological maturity	TSS (0 B)	Seeds/berry
1	826	30 Gy	3.8	30	19*	5.0	2.8	5.02*	324.0*	135	17.5*	16.0*	240*	124	16	2
2	828	30Gy	4.0	19	13	4.5	2.5	3.20	111.5	82	14.0	12.0	136	121*	20*	0*
3	831	30Gy	3.5	44*	18	6.0	3.5	5.16*	233.7	127	18.0*	15.0	184*	124	14	2
4	835	30Gy	3.9	24	17	6.5*	4.5*	3.17	232.3	121	17.0	14.5	192*	124	19*	1*
5	841	30Gy	3.4	15	18	5.0	2.8	2.80	320.0*	160*	17.0	14.0	200*	124	16	1*
6	870	30Gy	4.2	23	18	6.0	2.7	3.29	286.0*	143*	18.0*	16.0*	200*	121*	18	2
7	886	40Gy	4.4*	40*	19*	6.0	2.5	0.78	246.4*	140	17.5*	15.5	176	124	16	2
8	887	40Gy	5.2*	21	15	5.5	2.3	4.95*	137.8	82	18.0*	15.0	168	121*	18	2
9	947	0.1 % EMS 4 hrs.	4.4*	62*	15	5.5	4.5*	7.80*	119.6	115	18.0*	16.0*	104	126	15	2
10	949	0.1 % EMS 4 hrs.	3.9	47*	17	5.0	3.8	7.60*	153.6	120	17.0	15.0	128	124	17	1*
11	960	0.1 % EMS 4 hrs.	4.5*	35	20*	6.0	3.5	5.72*	159.0	142*	16.5	14.5	112	124	17	2
12	974	0.1 % EMS 4 hrs.	4.1	28	19*	7.0*	2.0	4.63	300.0*	150*	16.5	14.5	200*	124	19*	2
13	975	0.1 % EMS 4 hrs.	3.9	42*	17	6.0	2.0	6.28*	194.6	128	17.5*	16.5*	152	124	16	1*
14	1033	0.1 % EMS 8 hrs.	4.6*	45*	20*	7.0*	3.5	6.44*	216.0	135	17.5*	16.5*	160	124	18	2
15	1098	0.1 % EMS 12 hrs.	4.2	30	17	5.2	3.5	1.58	197.8	103	17.5*	16.0*	192*	124	18	1*
16	1159	0.2 % EMS 4 hrs.	4.8*	36	21*	5.3	4.5*	2.24	326.4*	170*	17.5*	16.0*	192*	124	17	2
17	1177	0.2 % EMS 8 hrs.	4.4*	40*	21*	6.0	2.0	5.00*	244.8*	180*	17.0	15.0	136	124	20*	1*
18	1190	0.3 % EMS 4 hrs.	3.8	43*	19*	7.0*	3.0	4.50	160.8	134	17.5*	16.0*	120	124	19*	2
19	ARI516 Control		3.81	18	16	5.5	2.8	3.4	121.0	167	16.3	14.6	138.5	123.3	17	1.8

\*Significant at 5% level of significance

Mutant M 828 of 30 Gy, gamma radiation treatment, was recorded seedlessness in berry character. Huge variability was observed in the mutant population for many characters of commercial significance. In the present study, the selection was focused on seedlessness. As a result, one seedless mutant (M 828) was identified in 2017 and that was confirmed in the 2018 season. Yield levels are yet to be stabilized. Mother plant showing seedless berries will be multiplied and many cuttings will be obtained during October and April pruning for further experimentation. There is a need for multi-location trials for the confirmation of their yield and performance under variable agro-climatic zones.

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