# Effects of *in vitro* gamma irradiation on two grapevine cultivars (*Vitis vinifera* L.) [1])

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Summary: The acute $\gamma$-irradiation (0, 20, 30, 40 Gy) of two white wine grape varieties, Trebbiano Romagnolo and Albana, during *in vitro* proliferation was tested. Trebbiano R. had a higher *in vitro* proliferation rate, but also showed a higher number of cultures with vitrification and morphological abnormalities. Albana withstood a maximum dose of 30 Gy, while Trebbiano R. withstood the highest dose used.

Among the field planted vines, variants with shorter internodes were found in the first 2 years after planting.

Key words: tissue culture, irradiation, mutation, breeding, variety of vine, shoot, malformation, internode length.

## Introduction

Mutation breeding has been applied to grapevine seeds and somatic tissues, as to other fruit species, using both physical and chemical mutagens. After irradiation, two or more vegetative multiplications are required for the induced mutations to become evident (Lapins 1983).

By combining irradiation of proliferating *in vitro* cultures with micropropagation, the time required for vegetative multiplication can be reduced from 2-3 years to a few months. For this reason, the two techniques have been applied to two Italian white wine grape varieties, Trebbiano Romagnolo and Albana (*Vitis vinifera* L.). Both of these cultivars are important and well established in North Italy Po Valley, where their grapes are mainly used to produce appellation wines (VQPRD). These varieties normally require cane pruning since their basal bud fruitfulness is low. An improvement in their basal bud fertility would enable growers to adopt spur-pruned training systems. In addition, both cultivars have very long internodes, while short internode variants, with more erect growth habit, would also be desirable. Finally, Trebbiano R. has tight bunches and looser bunches would decrease the incidence of the bunch rot in years of adverse weather conditions.

## Material and methods

### Culture establishment

*In vitro* cultures of the two wine grape cultivars Trebbiano R. and Albana were initiated in the early spring (1985). Individual buds were aseptically excised from uninodal cuttings that were collected in the field while still dormant and forced in greenhouse.

The standard proliferation medium (SPM) was composed as following: Murashige and Skoog (1962) mineral salts and vitamins (MS) with the addition of 2 mg/l benzylaminopurine (BA), 30 g/l sucrose, 7 g/l agar (Bacto-Difco). The pH was adjusted with 0.1 N KOH to 5.7 before autoclaving for 20 min at 120 °C.

Initially, the subculture interval was 1 month for both cultivars. Cultures were maintained in a growth room at a temperature of 23 ± 1 °C, 16/8 h (light/dark) photoperiod with a PPFD of 30 $\mu$mol $m^{-2}s^{-1}$. The explants were at first individually grown in test tubes (20 x 150 mm) and later

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transferred into 500 mm glass jars with metallic screw caps which were filled with 70 ml of medium and 10 cultures each.

To improve the unsatisfactory proliferation results of Trebbiano R., the subculture interval was shortened from 4 to 2 weeks and the SPM modified by using 1) different hormone combinations and 2) a double layer culture system with the MS salts of the liquid medium reduced to half and BA 0.5 mg/l applied over solid medium.

### Irradiation

Irradiation was performed in cooperation with the FARE Department of ENEA (Casaccia, Rome) by applying $\gamma$-rays from a $^{60}Co$ source. The following acute treatments (varied length of jar exposition, 1.53 Gy/min) were applied to proliferating and still actively growing cultures in 500 ml glass jars: control (no irradiation), 20, 30, and 40 Gy. Cultures were irradiated 10 d (Trebbiano) and 20 d (Albana) after transplant on SPM.

### Vegetative multiplication after irradiation

The cultures were transferred to fresh SPM 2 d after irradiation. A different protocol was then followed for the two cultivars.

Albana: When irradiated tufts were first subcultured (MVO-MV1; Lacey 1984), the longer shoots (> 15 mm) were individually placed in test tubes on SPM (to induce basal buds proliferation) and the shorter ones on a medium having the same composition but without hormones (to obtain shoot elongation). In the subsequent two subcultures only SPM was used.

Before transfer on the rooting medium, the shorter shoots were placed for one subculture on an elongation medium having the same composition as the SPM but with BAP reduced to 0.2 mg/l.

Trebbiano R.: In the first subculture after irradiation (MV1) all shoots, irrespective of their length, were placed in 500 ml jars on SPM medium. Unlike Albana, a 5 mm layer of liquid medium (composition cited above) was added on the top of the gelified medium to ensure proper nutrition. No elongation treatment was applied to short shoots since we had observed in previous trials that a high BA concentration was essential for satisfactory growth.

In proliferation, at the end of each subculture, the following data were recorded for both cultivars:

1) Number of living and dead cultures.
2) Proliferation rate (final/initial number of shoots), total length (mm) and node number of each culture.
3) Number of green, yellow, brown and vitreous cultures.
4) Number of normal and morphologically abnormal (leaf margin and/or blade) cultures.

After counting, brown and vitreous shoots were discarded as well as many shoots of the control to prevent an excessive number of control vines.

The MV3 shoots (fourth division after irradiation) were transferred on rooting medium having the following composition: MS mineral salts (reduced to half-strength and vitamins, naphthalene acetic acid (NAA) 0.5 mg/l, sucrose (commercial) 20 g/l, agar (Japan, unlabelled) 6.5 g/l.

The rooting phase *in vitro* occurred in the same growth room as the previous proliferation phase. After 20 d, the rooted cuttings were counted and transferred to soil (weaning), the dead cultures counted and discarded, and the unrooted cuttings placed on a fresh rooting medium after a renewal of the basal cut. After a further 20-d rooting period, the counting was repeated and the results expressed as the sum of the two rooting cultures.

### Soil establishment and field evaluation

Rooted cuttings were transferred to a peat, sand and perlite (1 : 1 : 1) mixture in plastic containers in a growth room at 23 ± 1 °C and PFD 100 $\mu$mol $m^{-2}s^{-1}$ where the relative humidity was

kept as high as possible for the 1st week. 40 d after transfer, culture survival was expressed as percentage of the initial number of plantlets. At the end of one full growing season in a nursery bench (1987), the developed plants were transferred to the field and cut back to two buds. The next winter (1988-89), the vines were again cut back to two buds. At the end of August 1988 and 1989, total shoot length (m) and number of nodes were recorded for each field planted vine.

Data concerning rooting and soil establishment were statistically analyzed with chi-square test, and linear regression calculated for the association shoot length and internode number of field-grown vines.

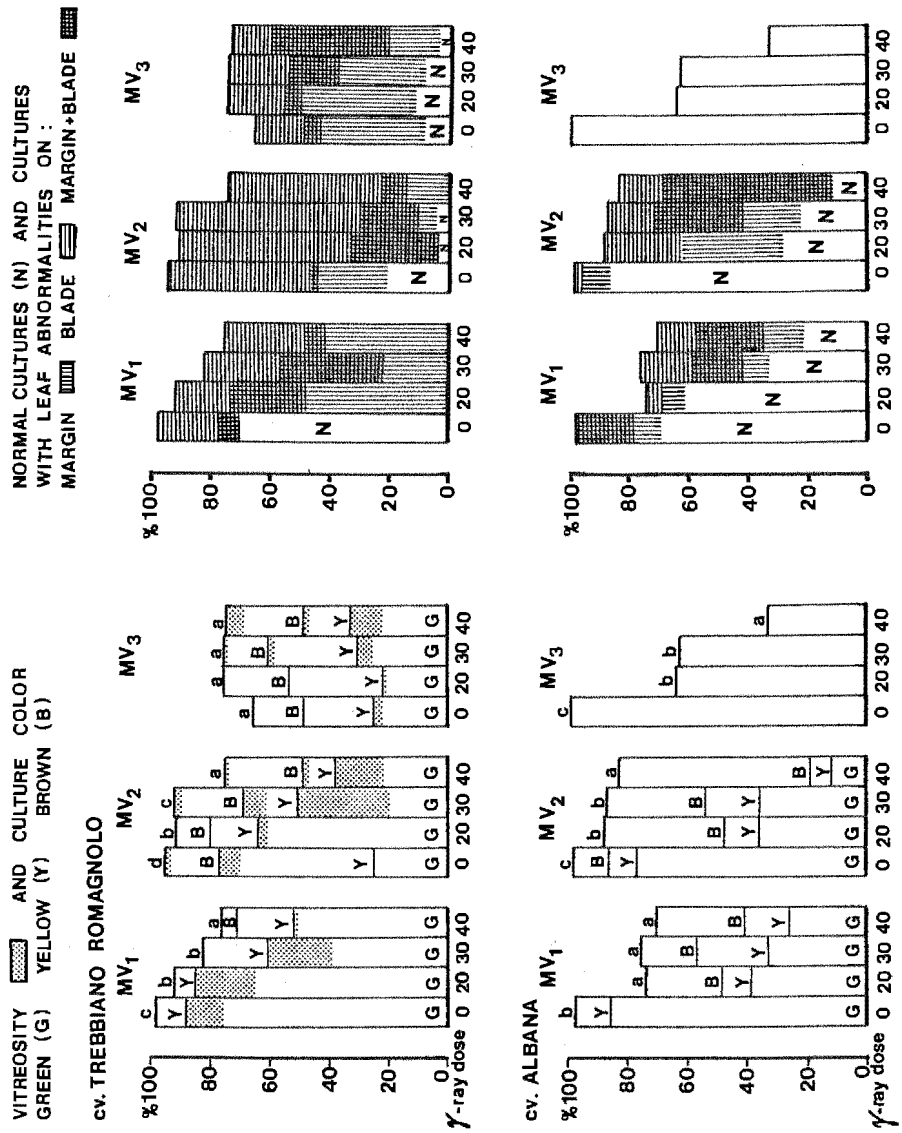


Fig. 1: Survival in the first three subcultures (MV1-MV2-MV3) after irradiation.

## Results

The *in vitro* cultures of Trebbiano R. had more problems than those of Albana. 15 months from initial explant were needed to obtain a sufficient number of uniform green Trebbiano R. shoots for irradiation and only 6 months for Albana. SPM induced the best proliferation and survival for both cultivars, however, the subculture interval for Trebbiano R. had to be reduced from 4 to 2 weeks.

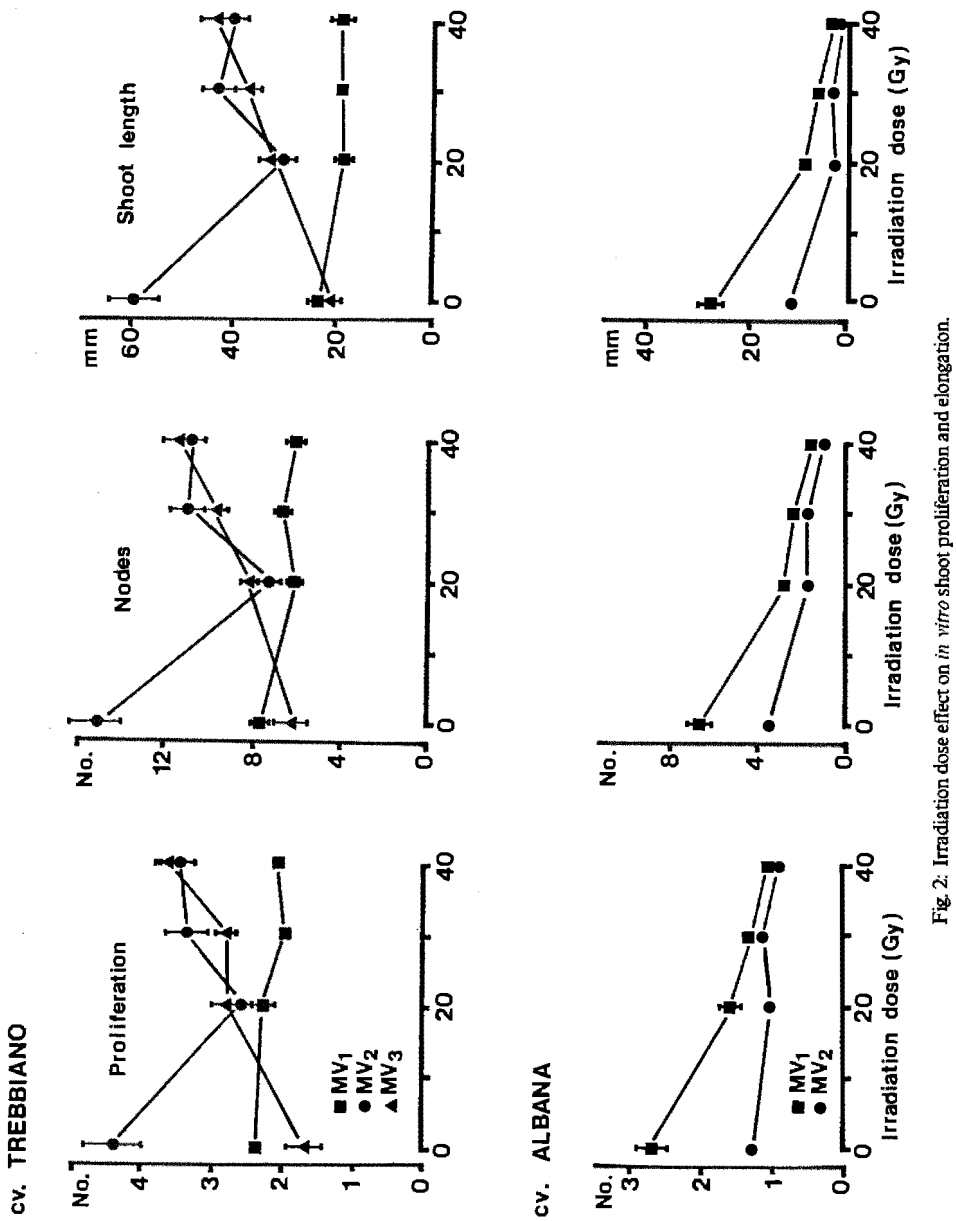


Fig. 2: Irradiation dose effect on *in vitro* shoot proliferation and elongation.

Irradiation dose effect on *in vitro* shoot proliferation and rooting. Vines transferred to weaning, nursery and field

| γ-ray dose Gy | In-vitro shoots MVO No. | MV3 No. | Rooted % | Vines transferred to: Weaning No. | Nursery No. | Field 1988 No.(z) |
|---|---|---|---|---|---|---|
| cv.TREBBIANO ROMAGNOLO | | | | | | |
| 0 | 154 | 45[y] | 100 c | 45 | 36 | 19 |
| 20 | 93 | 234 | 86 a | 202 | 87 | 21 |
| 30 | 106 | 235 | 92 a | 215 | 48 | 0 |
| 40 | 72 | 177 | 98 b | 173 | 107 | 23 |
| cv. ALBANA | | | | | | |
| 0 | 50 | 85[y] | 100 b | 85 | 68 | 16 |
| 20 | 252 | 114 | 72 a | 82 | 54 | 54 |
| 30 | 194 | 132 | 67 a | 88 | 52 | 52 |
| 40 | 166 | 13 | 61 a | 8 | 7 | 7 |

Mean separation within cultivars, chi-square test, P=0.05

(y) Only a few of the control shoots were maintained

(z) Several Trebbiano R. vines were field planted only in 1989

## Proliferation and survival

Fig. 1 shows the effects of irradiation. Culture survival decreased as the doses increased except in Trebbiano MV3; the phenomenon was particularly evident in Albana MV3.

Vitrification occurred in all Trebbiano R. treatments and subcultures, but was not present in Albana. Leaf browning appeared in MV1 in the latter cultivar and its percentage increased in MV2 particularly at 40 Gy. In contrast, leaf browning was found in Trebbiano R. starting from MV2 with no differences between irradiation doses.

Abnormalities in leaf margin and/or blade were higher in Trebbiano R. (reaching 100 % in irradiated MV1 material). In Albana, abnormalities increased along with doses and from MV1 to MV2 (Fig. 1).

In light of the proliferation and shoot elongation data, the different response to irradiation and tissue culture of the two cultivars was evident (Fig. 2). Proliferation in Trebbiano R. was higher than in Albana, which later showed a constant decrease of values from control to Gy 40. Irradiation had a depressive effect in Trebbiano R. MV1 and MV2, while in MV3 it stimulated proliferation.

Mortality, shoots discarded for browning and low proliferation rate, lowered the final number of transferred Albana shoots below the initial one, particularly at Gy 40. In contrast, the final number of Trebbiano R. shoots was higher than the initial number, mainly because of the high proliferation rate, irrespective of the irradiation dose (Table and Fig. 2).

Irradiation, as expected, decreased rooting in both cultivars, but its effect was less marked in Trebbiano R. Albana had the best weaning results as shown by the higher percentage of vines transferred to nursery (Table).

## Field growth

Some variants (4 of Trebbiano R. and 4 of Albana) for vegetative characters were found in vine populations in 1988, 5 of them with longer and 3 with shorter internodes. In 1989, only variants with shorter internodes were observed (1 in Trebbiano R. and 6 in Albana); 1 of them, in Albana, performed exactly as in the 1st year. In 1989, the vines showed longer shoots because of longer internodes as compared to 1988 (Fig. 3).

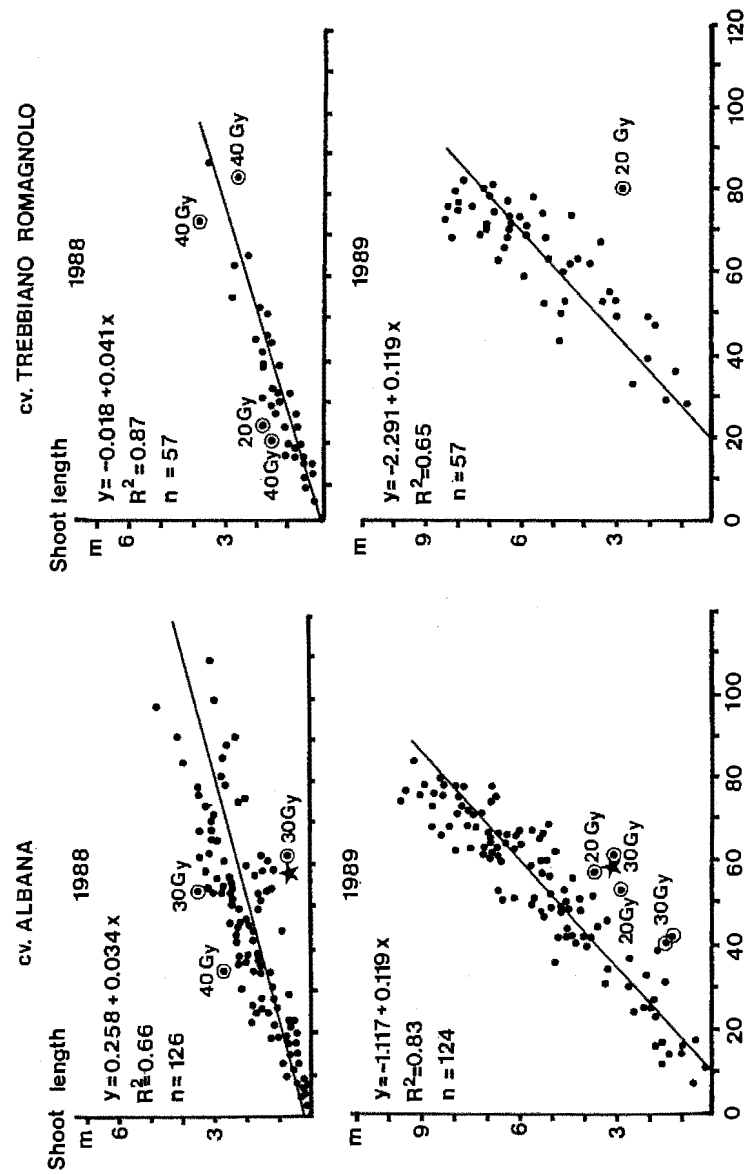


Fig. 3: Correlation between number of nodes and shoot length for field-grown Albana and Trebbiano R. grapevines in 1988 and 1989.

## Discussion and conclusions

The response of the two grapevine cultivars to tissue culture on the same medium was different and not completely satisfactory. Trebbiano R. had a good proliferation rate which should have meant a successful *in vitro* propagation. But the vitreousity, leaf yellowing or browning and morphological abnormalities clearly indicated that the adopted medium can be improved. For example, vitrification has been linked to metabolic perturbations induced by chemical or physical factors related to medium composition (DEBERGH *et al.* 1981; PAQUES and BOXUS 1987; PASQUALETTO *et al.* 1988). In addition, Albana evidenced low proliferation rate which may be enhanced by increasing the cytokinin concentration as is done in other grape cultivars (HARRIS and STEVENSON 1982).

The two tested cultivars also proved to have a different sensitivity to irradiation: Albana withstood a maximum dose of 30 Gy while Trebbiano R. withstood the highest dose used (40 Gy). These results concur with the findings on irradiation *in situ* of dormant grapevine material in which doses ranging from 50 Gy (REICHARDT 1955) to 30 Gy (DONINI 1975) were suggested. *In vitro* irradiation of grapevine required a lower or similar dose than that applied *in situ*, as suggested for other top fruit species (LACEY and LENNARD 1977; PREDIERI *et al.* 1986).

Despite the difficulties encountered, the techniques employed yielded in a short time weaned and field-transferred grapevines (January 1988). In these plants, the only vegetative trait that could be considered during 1988 and 1989 was the internode length. The first season after transplant to the field, variants with longer and shorter internodes were found. The former disappeared the following season.

A variant of Albana, isolated from 30 Gy irradiated material, exhibited shorter internodes in both seasons, differing significantly from the mean of the population observed. Further observations of this and of the other variants will be needed over the next few years to confirm these preliminary results. Fruiting of the irradiated vines in 1990 will also allow the evaluation of productive traits.

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