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Research Article

Reactions of Some Grapevine Rootstock Cuttings to Mutagenic Applications

Zeki KARA[1,*], Osman DOĞAN[1],

[1]Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, Türkiye

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ABSTRACT

Grapevine (*Vitis* spp.) is one of the most important socio-economically important plants in the global scale, and the need for its breeding is increasing. In viticulture, mutation is more promising than crossbreeding in breeding new genotypes from natural genetic diversity. Polyploid plants outperform their diploid relatives in several respects. In this study, the efficacy of oryzalin and N_2O mutagens in the induction of polyploidy was investigated by applying different doses and durations to the forced cuttings of 41B [Chasselas (*Vitis vinifera* L.) × *Vitis berlandieri* Planch)] and Fercal [(*Vitis vinifera* x *Vitis berlandieri*) × 333 EM] rootstocks. LD_{50} values of mutagen applications were determined, morphological and cytological effects were examined by macroscopic, microscopic, and cytological methods. Application time and dose increase of mutagens decreased LD_{50} values. As a result of mutagen applications, leaf thickness and chlorophyll content of the surviving plants increased. Applications increased stomatal sizes, decreased their density, increased chloroplast numbers, increased leaf thicknesses and partially SPAD values. It was determined that they were not polyploid in the confirmation test performed with flow cytometry (FC) analyses in 4 Fercal and 1 41B samples that were assumed to be mutant by stoma and chloroplast examinations. After that, it was thought that it would be appropriate to try vegetative material with actively dividing cells, such as nodal cuttings, in the studies of obtaining polyploid individuals on grapevine rootstocks

1. Introduction

Grapevine (Vitis spp.) is one of the most important socio-economically important plants globally due to the diversity of the products obtained, and there is an increasing demand for its breeding. 77 million tons of grapes are produced from an area surface of 9.9 million hectares in the world. While China ranks first in grape production with 17372167 tons, Turkey ranks sixth with 4.1 million tons of production (Faostat, 2021). The grape market, which was 68 billion dollars in 2016, is the most money-making horticultural crop in the world after tomato (Alston and Sambucci, 2019).

It is thought that the vine has the most variety when compared to other cultivated plant species. In the world, 25538 grapevine genotypes and 1432 grapevine rootstocks are recorded in the Vitis Database (VIVC, 2021). While approximately 10 grapevine rootstock varieties are used in 90% of vineyards worldwide (Keller, 2020), Teleki/Kober selection rootstocks probably constitute 50% of them (Reynolds, 2015). On the other hand, there are many vineyard regions or locations around the world in different climates and different soils. The few rootstock varieties currently used are unlikely to meet the requirements of all viticulture areas (Reynolds, 2015).

Traditional grape breeding poses great challenges to breeders due to the complexity of the traits and the long breeding process of approximately 25 years (Töpfer et al., 2011).

Polyploidy is the presence of more than two genomes per somatic cell. Generally, the polyploid organism has more than one set of chromosomes, or a combination of sets of chromosomes found in the same species, or a closely related diploid species. Polyploid organisms can arise spontaneously (mitotic ploidy) with chromosome copies of somatic cells or in meiosis, with non-segregation of homologous chromosomes giving rise to diploid gametes (Ramsey and Schemske, 2002).

Polyploidy has played an important role in the evolution of higher plants (Leitch and Bennett, 1997). Originally polyploids were thought to have a single origin and assumed genetic uniformity among all individuals of a species. Application of recent molecular biology techniques, particularly DNA markers, has shown that a single polyploid species can have multiple origins,

* Corresponding author email: *zkara@selcuk.edu.tr*

suggesting that there is more variation in polyploids than single origin (Soltis and Soltis, 1993; Soltis et al., 1993; Leitch et al. Bennett, 1997; Soltis and Soltis, 1999; Doyle et al., 2004; Soltis et al., 2004; Martelotto et al., 2007).

Successful promotion of polyploidy requires a synergistic coupling of effective penetration of the antimitotic agent and may depend on the exposure time and doses of antimitotic agents, tissue types, basal environment, and interactions with plant growth regulators (Touchell et al., 2020). With the right application time and duration, it can easily penetrate the plant due to the small molecule structure of N_2O and promote polyploidy. In addition, N_2O is not as harmful to nature as colchicine (Kara et al., 2018c).

In this study, the morphological and cytological effects of chemical mutagen applications with the applications of oryzalin and N_2O on the cuttings of 41 B and Fercal rootstocks with different doses and durations to meet the global grapevine rootstock requirement were investigated by macroscopic, microscopic, and cytological methods.

2. Materials and Methods

Cuttings containing at least three buds, taken from 41 B [Chasselas (*Vitis vinifera* L.) × *Vitis berlandieri* Planch)] and Fercal [(*Vitis vinifera* x *Vitis berlandieri*) × 333 EM] rootstocks during the resting period and kept in an airtight bag at +1 °C, were used as plant material. The cuttings were placed in pressure resistant tanks and gas flow was provided to the tank through the pressure regulator connected N_2O tube. Various doses (0, 2.5, 5 and 10 bar) and durations (48h-96h) of N_2O were applied to the cuttings with cell division in the phenological development stages 3-5 of Eichhorn and Lorenz (1977). N_2O applied cuttings were planted in cutting rooting pans (peat: perlite, 3:1). For the orzalin application, the cuttings were planted in the pans, 0, 2.5, and 100 µM at the same growth stage were applied twice a day (at 08:30 and 18:00), followed by 48 hours (48h) and 96 hours (96h).

In surviving plants, stomatal density per unit area (number mm^{-2}) (Kara et al., 2018c), stomatal sizes (µm) (Kara et al., 2018a), chloroplast numbers in stoma guard cells (number $stoma^{-1}$) (Kara et al., 2018a; Yazar, 2021), leaf thickness, chlorophyll content (Kara et al., 2018b) and Flow Cytometry (FC) analyzes (Yazar, 2018) were done. According to the results of all analysed data, the effects of oryzalin and N_2O treatments on chromosome folding in grapevine were evaluated.

Fresh leaf samples (3-4 weeks) were taken for FC analysis from plants that survived after oryzalin and N_2O application and whose ploidy level was predicted by chloroplast counts. Sections of 0.5 cm^2 were taken from the leaf samples, placed in petri dishes, and 500 µL of isolation buffer (Partec-Nuclei Extraction Buffer) was added and the leaf tissue was cut into small pieces with a razor blade. As a result of the lysis process by adding isolation buffer, the cell nuclei were released, and openings were formed on the nuclear membrane. The samples in the Petri dish were shaken for 10-15 seconds and transferred to tubes (Partec-Sample Tubes, 3.5 ml, 55×12 mm) filtered through a Partec-CellTrics 30 µm-green filter. 1600 µL of staining solution [Partec-DAPI (4,6 diamidino-2-phenylindole) Staining Buffer] was added to the tubes, incubated for 5 minutes in a light-isolated environment. Afterwards, the samples were analysed in the FC device (Yazar, 2018).

The data obtained because of oryzalin and N_2O treatment of 41B and Fercal cuttings were compared with the Duncan multiple comparison test in the IBM SPSS 17.0 statistical program (SPSS Inc, Chicago, IL, USA) at $p<0.05$ significance level (Yue et al., 2017).

3. Results and Discussion

3.1. Survival rate (%)

The effects of oryzalin and N_2O applications on 41B and Fercal cuttings were significant on the viability rate (Figure 1). Viability rates of 41B cuttings treated with oryzalin were 83.33±5.77%, 46.67±5.77%, 28.33±2.89%, 30.00±5.00 and 11.67±2.89% for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h, respectively and the same values were determined in Fercal as 86.67±5.77%, 50.00±5.00%, 46.67±2.89%, 40.00±5.00% and 26.67±2.89%.

The viability rates of N_2O applied 41B for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h applications were 83.33±5.77%, 38.33±2.89%, 25.00±5.00%, 26.67±5.77%, 16.67±2.89%, 21.67±2.89% and 6.67±2.89%, respectively, and in Fercal they were listed as 86.67±5.77%, 46.67±2.89%, 21.67±7.64%, 31.67±2.89%, 13.33±2.89%, 15.00±5.00% and 6.67±5.77%, in the same order. The highest viability of both rootstock cuttings was determined in the control, while the lowest was determined in the application of 10 bar 96h N_2O applications.

Antimitotic agents, especially applied at high concentrations, generally inhibited plant survival (Zakizadeh et al., 2020). In previous studies, oryzalin (Xie et al., 2015) and N_2O (Molenaar et al., 2018) reduced the survival rate. Some researchers have found oryzalin to be highly toxic to plants, especially at higher concentrations and longer exposure times (Dunn and Lindstrom, 2007; Dhooghe et al., 2009). In general, lower concentration and shorter application times decreased the acquisition frequency of tetraploid plants while increasing the survival rate (Chakraborti et al., 1998; Väinölä, 2000; Zhang et al., 2008).

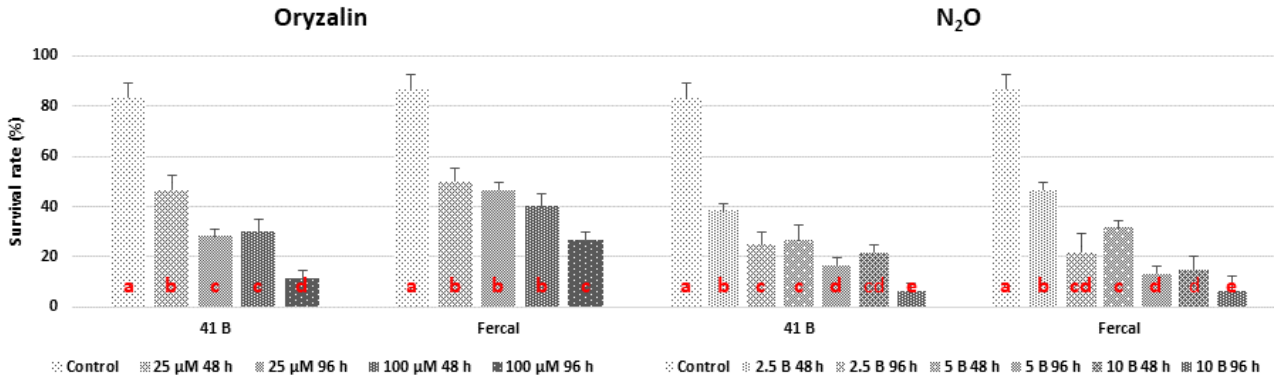

Figure 1
Mutagen effects on the surviving rate of 41 B and Fercal rootstocks

3.2. Average lethal dose (LD_{50})

The LD_{50} values for 48h and 96h were 18.95 µM and 4.12 µM, respectively, in oryzalin applications to 41B. LD_{50} values were recorded as 25.00 µM and 19.84 µM for 48h and 96h, respectively, in the oryzalin applications to Fercal. LD_{50} values decreased with increasing time in applications made to both rootstock cuttings (Figure 2a). The LD_{50} values of N_2O applications were determined as 0.86 bar and 0.39 bar for 48h and 96h applications to 41B cuttings, respectively. The LD_{50} values of N_2O applied Fercal for 48h and 96h were 2.19 bar and 0.18 bar, respectively. In the oryzalin applications, the increase in time decreased the LD_{50} value (Figure 2b).

The LD_{50} values of oryzalin applied 41B were 42.32 hours and 22.53 hours for 25 µM and 100 µM, respectively, and the same values for Fercal were 48 hours and 7.13 hours for 25 µM and 100 µM. In the oryzalin applications, the LD_{50} values were decreased in contrast to the dose increase (Figure 2c).

The LD50 values obtained with N_2O applications to 41B were 26.16 hours, 9.52 hours, and 7.96 hours for 2.5, 5, 10 bar, respectively, and the LD_{50} values in Fercal were 43.75 hours, 24 hours, and 2.61 hours for 2.5, 5 and 10 bar, respectively. Contrary to the increase in N_2O dose, LD_{50} values in the durations decreased in both grapevine rootstocks (Figure 2d).

The mean lethal dose (LD_{50}) is often used as a critical parameter for chemical mutagens (Chen et al., 2020). LD_{50} studies evaluate the susceptibility of a mutagen-treated plant part of a particular cultivar to a mutagen (Jain, 2010; Cabahug et al., 2020). Therefore, a series of dose tests is performed to determine which concentration will provide 50% regeneration survival. A series of doses applied in constructing a dose-response curve by examining the data is termed "optimal", where LD_{50} doses and beneficial mutants are reported (Szarejko, 2012).

In previous studies, the LD_{50} value was calculated in two ways. In the first, applications with a 50% mortality rate are given as the LD_{50} value (Asoko et al., 2020; Cabahug et al., 2020; Pehlivan, 2020). In the second, lethal dose-based linear regression was used (Pal et al., 2017; Chen et al., 2020). Time- and dose-dependent LD_{50} value was determined by us by lethal dose-based linear regression method. In our study, LD_{50} values decreased as in previous studies, as in previous studies, in contrast to the application time or dose increase (Kerdsuwan and Te-chato, 2012; Mahajan et al., 2015; Pal et al., 2017; Chen et al., 2020; Hasim et al., 2021).

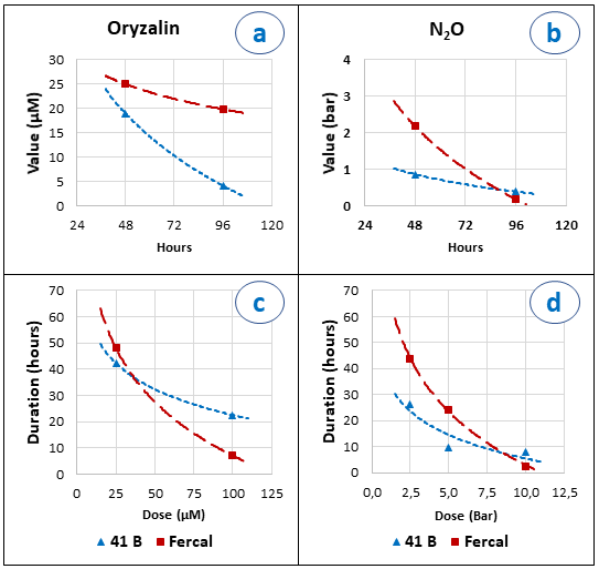

Figure 2
Effects of oryzalin and N_2O treatments on time (a, b) and dose (c, d) dependent LD_{50} values on 41 B and Fercal vine rootstocks.

3.3. Stoma density (number mm^{-2}) and stoma sizes (µm)

The effects of oryzalin and N_2O application on the number of stomata in 41B and Fercal were significant. The number of stomata of oryzalin applied 41B were 196.46±2.94, 184.98±6.69, 179.11±3.50, 173.62±3.14, 170.92±2.34 for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h, respectively, while it was determined as 196.62±3.82, 187.84±4.63, 173.08±3.50, 173.17±2.71 and 169.61±1.62 units in Fercal, in the same order. In both rootstocks, the densest stomata were detected in the control group, and the lowest density was detected in the application of 100 µM for 96h (Figure 3a).

In N_2O applied 41B, stoma densities were 196.46±3.17, 191.22±2.05 for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h,

respectively, and in Fercal that were 186.79±2.90, 183.49±3.80, 177.27±2.23, 174.92±1.98, 171.08±2.43 were determined as 196.62±3.05, 190.76±2.28, 187.62±2.85, 184.31±2.16, 178.65±2.59, 174.36±2.13 and 171.35±1.61, in the same order. In both rootstocks, the most stoma number was determined in the control, while the least stoma was recorded in the 10 bar 96h application (Figure 3a).

Stomatal lengths in the oryzalin treated 41B were 26.02±0.36 µm, 26.29±0.16 µm, 26.85±0.09 µm, 27.30±0.27 µm, and 27.75±0.25 µm for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h applications, and in Fercal that were 26.07±0.24 µm, 26.40±0.55 µm, 26.94±0.19 µm, 28.05±0.17 µm, and 28.53±0.33 µm, in the same order. The highest value was determined at 100 µM 96h in both rootstocks, and the lowest in the control (Figure 3b).

In the N_2O treated 41B, stomatal lengths were 26.02±0.36 µm, 26.34±0.42 µm for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h, 26.61±0.50 µm, 26.94±0.19 µm, 27.48±0.33 µm, 27.81±0.33 µm and 28.22±0.36 µm, respectively. In the Fercal that were 26.07±0.24 µm, 26.40±0.37 µm, 26.73±0.22 µm, 27.08±0.34 µm, 26.91±0.09 µm, 27.31±0.18 µm and 28.12±0.31 µm, respectivelly. The longest stoma was determined in the 10 bar 96h application in both rootstocks, while the lowest stoma was recorded in the controls (Figure 3b).

Effects of oryzalin applications on stomatal width in 41B were recorded as 18.15±0.29 µm, 18.52±0.61 µm, 18.90±0.19 µm, 19.06±0.42 µm, and 18.91±0.27 µm for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h, respectively while in Fercal these data were determined as 18.40±0.43 µm, 18.40±0.41 µm, 19.17±0.40 µm, 18.93±0.43 µm and 19.31±0.14 µm in the same order. The largest stomata was in 41B at 100 µM 48h application, and in Fercal 100 µM 96h application. The narrowest stoma was detected in the control in both rootstocks (Figure 3c).

The effects of N_2O applications on stomatal widths in 41B were measured 18.15±0.29 µm, 18.33±0.37 µm, 18.26±0.24 µm, 18.66±0.15 µm, 18.93±0.20 µm, 18.67±0.48 µm, and 19.45±0.28 µm for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h, respectively while in Fercal these values were 18.40±0.43 µm, 18.20±0.29 µm, 18.46±0.25 µm, 18.32±0.18 µm, 18.98±0.24 µm, 19.07±0.26 µm and 19.33±0.45 µm, respectively. The smallest stomata width was detected in 41B control, and in Fercal 2.5 bar 48h application, and the widest at 10 bar 96h in both rootstocks (Figure 3c).

Stoma sizes are an indirect method for identifying polyploids (Moghbel et al., 2015). In general, stomatal characteristics were used for rapid and early identification of polyploids (Cohen and Yao, 1996; Gu et al., 2005; Tang et al., 2010). In addition, the detection of stoma features is simple and does not require expensive instruments. Xie et al. (2015), the most economical and efficient methods for determining the ploidy level are the determination of stomatal sizes and chloroplast numbers, but it was suggested that this method should be applied together with other modern methods for definitive results (Huy et al., 2019).

The variation in stomatal size is one of the most remarkable advanced agronomic features of tetraploid plants (Kosonoy-González et al., 2019). Polyploid plants have larger stomatal sizes and lower stomatal density per unit area than diploid plants (Yang et al., 2006; Lu et al., 2014; Kara et al., 2018c; Bae et al., 2020). This difference is probably due to increased cell size of polyploid plants (Marinho et al., 2014) and/or decreased leaf mesophyll space (Lundgren et al., 2019). Although the stomatal characteristics of the selected plants in our study were like the results of previous studies (Kara et al., 2018a; Zeng et al., 2019; Bae et al., 2020), ploidy could not be confirmed.

3.4. Leaf Thickness (µm)

The effects of mutagen applications on the leaf thickness of 41B and Fercal cuttings at different doses and times were significant. The leaf thicknesses of oryzalin applied 41B were 137.54±5.13 µm, 149.09±4.64 µm, 153.20±3.64 µm, 145.54±4.19 µm, and 156.09±4.83 µm for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h, respectively. Leaf thicknesses of Fercal were measured as 136.58±5.00 µm, 134.70±4.14 µm, 150.61±3.55 µm, 153.43±4.48 µm and 159.12±2.25 µm in the same order. In both genotypes, the thickest leaves were detected in 100 µM 96h application, while the thinnest leaves were determined in 41B in control and in 25 µM 48h in Fercal (Figure 4a).

The leaf thicknesses of N_2O applied 41B were 137.54±5.13 µm, 137.68±3.47 µm were 138.60±3.69 µm, 134.80±3.38 µm, 152.35±2.73 µm, 155.91±2.13 µm and 148.90±4.09 µm for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h, respectively, and in the Fercal taht were determined as 136.58±5.00 µm, 140.88±3.24 µm, 136.18±3.75 µm, 138.33±3.22 µm, 144.65±5.04 µm, 142.60±4.58 µm and 151.10±5.24 µm in the same order. As a result of N_2O applications, while the thickest leaves were determined in the application of 10 bar 48h in 41B, the thinnest leaves were detected in the 5 bar 48h hours. In Fercal, the thickest leaves were recorded in 10 bar 48h, the thinnest leaves in 2.5 bar 96h application (Figure 4a).

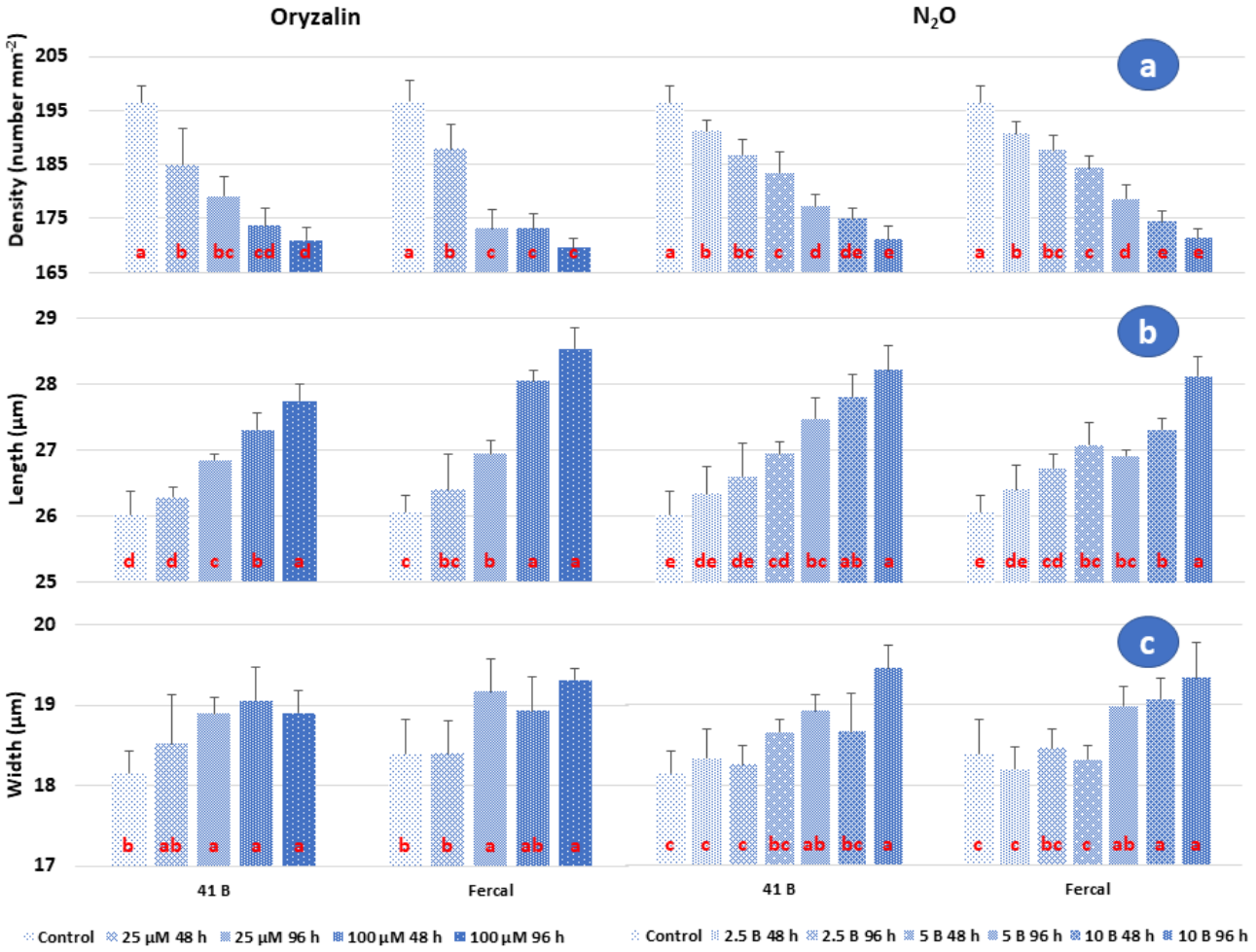

Figure 3
Mutagen effects on stomatal density (a), length (b), and width (c) on 41 B and Fercal rootstocks

Tetraploid plants have wider and thicker leaves than diploids (Rao et al., 2019). In previous studies (Lu et al., 2014; Zeng et al., 2019), leaf thickness of tetraploid plants obtained with oryzalin applications increased compared to their diploid origin. Bae et al. (2020) reported that the leaves of tetraploid plants are smaller, thicker, and wrinkled than their diploids.

3.5. SPAD Value

The differences between the chlorophyll content data obtained by the mutagen applications to 41B and Fercal were significant. Chlorophyll content data of oryzalin in 41B was 27.02±0.26, 27.35±0.16, 28.13±0.30, 28.83±0.46 and 32.59±0.33 for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h, respectively, and in Fercal that were 27.62±0.57, 25.87±0.12, 27.87±0.10, 28.42±0.42, and 32.78±0.52, respectively. In both rootstocks, the highest chlorophyll content was recorded in 100 µM 96h application, the lowest chlorophyll contents in 41B control and 25 µM 48h application in Fercal (Figure 4b).

Chlorophyll contents in the N_2O treated 41B were 27.02±0.26, 26.77±0.40, 26.29±0.27, 27.93±0.32, 31.73±0.27, 28.95±0.27 and 28.98±0.48 for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h, respectively, in Fercal these values were 27.62±0.57, 27.61±0.43, 27.82±0.42, 26.91±0.22, 31.09±0.34, 31.90±0.86 and 34.16±0.16, respectively. The highest chlorophyll content was determined in 41B at 5 bar 96h, in Fercal at 10 bar 96h, the lowest chlorophyll contents in 41B at 2.5 bar 96h, and at 5 bar 48h in Fercal (Figure 4b).

Herbicides cause whitening (photobleaching) of green tissues by inhibiting the carotenoid biosynthesis pathway, reducing the concentration of coloured carotenoids, and causing photodynamic destruction of chlorophyll molecules (Boger and Sandmann, 1998). On cellular leakage, oryzalin causes some loss of membrane integrity, which is more evident when exposed to light (Dayan and Watson, 2011). The effect of herbicides on plant cells can result in complete loss of microtubules and eventual cell death, not only during mitosis but also during interphase (Yemets and Blume, 2008).

Since herbicides cause photodynamic destruction of chlorophyll molecules and cause green tissues to whiten (photo bleaching), mutagen applications damage chlorophyll molecules (Boger and Sandmann, 1998). Tetraploid plants have higher chlorophyll content compared to diploids and high chlorophyll content can create high photosynthetic capacities (Eng and Ho, 2019).

It was reported in previous studies that the chlorophyll content of tetraploid plants increased compared to diploids (Rao et al., 2019; Mo et al., 2020) found to be significantly less than that of diploids. These differences are thought to be related to the species.

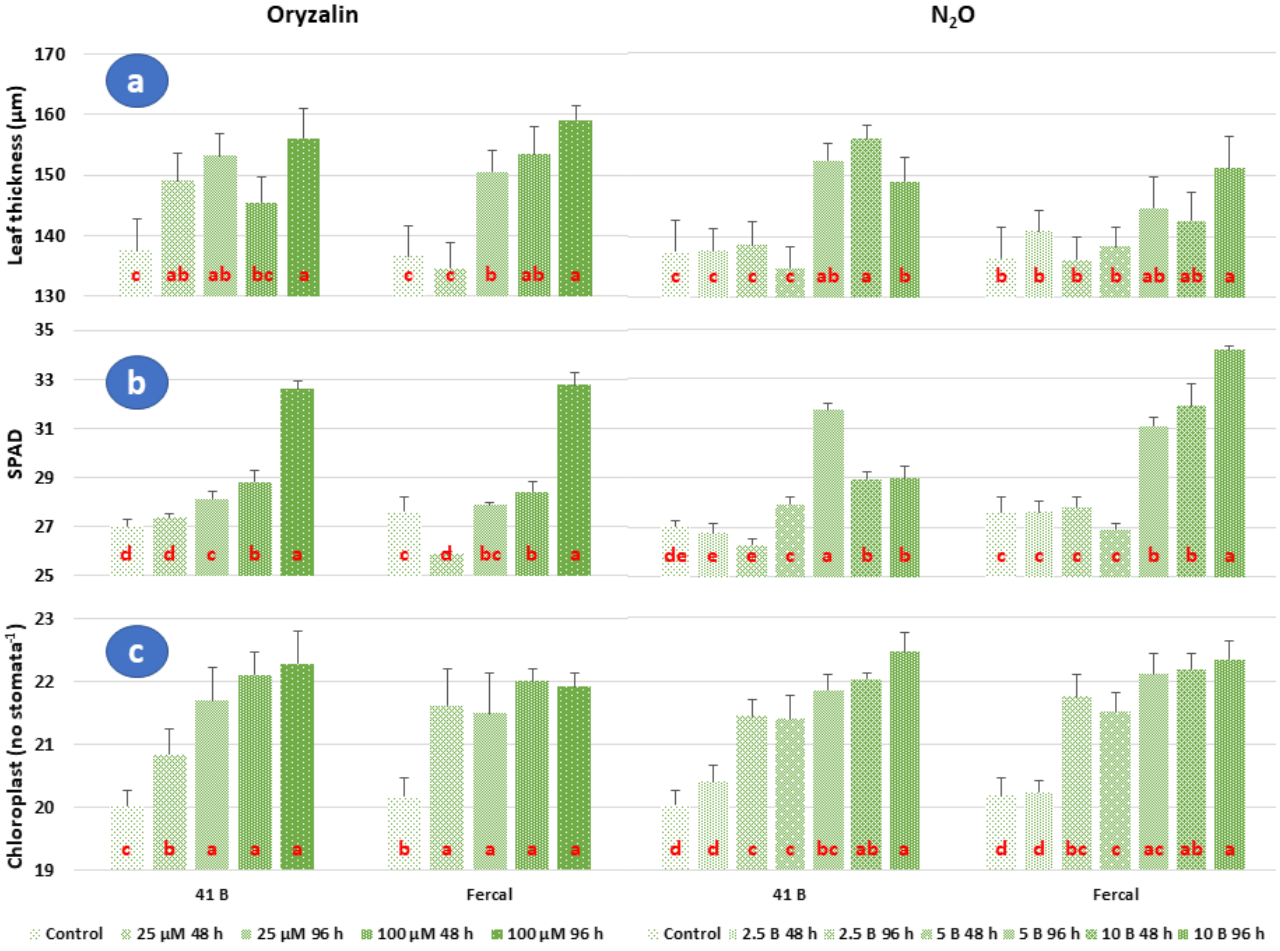

Figure 4
Mutagen effects on leaf thickness (a), SPAD (b), and chloroplast numbers (c) on 41 B and Fercal rootstocks

3.6. Chloroplast number (number stomata^{-1})

The effects of mutagen on 41B and Fercal on chloroplast numbers were significant. Chloroplast numbers in oryzalin applied 41B were 20.04±0.23, 20.85±0.40, 21.71±0.53, 22.11±0.36 and 22.30±0.50 for control, 25 µM 48h, 25 µM 96h, 100 µM 48h and 100 µM 96h, respectively. While these values were recorded as 20.18±0.29, 21.63±0.59, 21.50±0.64, 22.01±0.20 and 21.93±0.22 in Fercal, respectively. The least chloroplasts were detected in the control in both rootstocks. 100 µM 96h in 41B, and 100 µM 48h in Fercal caused the most chloroplast increase (Figure 4c).

Chloroplast numbers in the N_2O treated 41B were 20.04±0.23, 20.41±0.25, 21.45±0.27, 21.40±0.38, 21.85±0.26, 22.03±0.11 and 22.46±0.31 for control, 2.5 bar 48h, 2.5 bar 96h, 5 bar 48h, 5 bar 96h, 10 bar 48h and 10 bar 96h, respectively. That were determined in Fercal as 20.18±0.29, 20.24±0.18, 21.76±0.35, 21.51±0.32, 22.11±0.32, 22.18±0.25, 22.34±0.28, respectively. The lowest chloroplast numbers were detected in the control, and the highest chloroplast increase was detected in the 10 bar 96h application (Figure 4c).

Changes in the anatomical features of leaves associated with an increase in cell size in parallel with the increase in ploidy level in plants (Dwivedi et al., 1986) are widely used to evaluate and monitoring the polyploidy promotions (Zhang et al., 2010). In some previous studies, ploidy levels were evaluated according to stomatal length, width, area, and density (Dwivedi et al., 1986; de Carvalho et al., 2005; Yang et al., 2006). Some researchers have reported that the number of chloroplasts per stomatal guard cell is more effective in determining ploidy levels (Compton et al., 1996; Chakraborti et al., 1998; Zhang et al., 2005), however, chloroplast numbers in the stomatal guard cell were found to be similar in diploid and tetraploid plants (de Carvalho et al., 2005). These differences are probably due to the plant species used in the studies (Zhang et al., 2010).

3.5. Flow cytometry

Since the chloroplast counts showed polyploidy findings in only 5 plants (4 Fercal and 1 41B) from all treatments, FC analysis was performed to confirm the ploidy levels of these samples. As a result of FC analysis, polyploidy could not be confirmed in the genotypes examined. In FC analyses of diploid control plants and

mutagen treated plants, the peak level was determined around 200 (Figure 5).

FC analysis is a fast, reliable, and simple method to determine the ploidy level and confirm the success of polyploidy induction, and is one of the leading methods, enabling the analysis of large numbers of target plants in a short time (Roy et al., 2001; Dhooghe et al., 2011). Some researchers reported that FC analysis is the most effective and reliable method to detect changes in ploidy level (Dolezel, 1997; Loureiro et al., 2005; Sakhanokho et al., 2009).

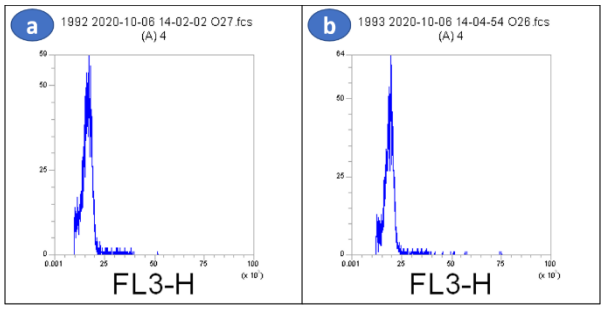

Figure 5
FC analysis result of diploid control plant (a) and mutagen treated and selected 41B sample (b)

4. Conclusion

No polyploid individuals could be obtained by *in vivo* applications of oryzalin and N_2O to the cuttings of 41B and Fercal rootstocks. However, LD_{50} values were determined for the effects of mutagens on grapevine rootstocks. These data will be a guide for future studies. Mutagen applications caused a decrease in stomatal densities, stomatal sizes, chloroplast numbers, leaf thickness and SPAD values of rootstock genotypes. Due to the strong DNA damage repair system in grapevine rootstock genotypes, it is thought that the formation of confirmed polyploid grapevine rootstocks is prevented despite preliminary polyploid data. However, to obtain polyploid individuals from vegetative material in grapevine genotypes, it is thought that it would be appropriate to try smaller vegetative shoot and root cuttings, or somatic embryos developed by somatic embryogenesis instead of large size cuttings.

5. Acknowledgements

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Conflict of interest

Z. Kara, O. Doğan, declare that they have no competing interests.

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