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Early water stress effects on pollen viability, berry set and embryo development in cv. 'Syrah' (*Vitis vinifera* L.)

Ilknur Korkutal[1], Elman Bahar[1], Alain Carbonneau[2]*

[1]Namik Kemal University, Agricultural Faculty, Department of Horticulture, Tekirdag, Turkey
[2]Agro M, Viticulture-Oenologie (retired), Montpellier Cedex, France

**Corresponding author: ikorkutal@nku.edu.tr*

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Abstract: Early water deficit at bloom results in poor berry set and/or a grape yield reduction. The purpose of this work was to determine the effects of early water stress in a berry's development. The experiment was carried out in a SupAgro ECOTRON System in Montpellier, France. Seven year- old 'Syrah' vines were used. The pot's surface was protected from rainfall, and the growing medium was perlite+coarse sand with controlled drainage. The treatments were WS_0 (control): 4 l/day, WS_1: 3 l/day, WS_2: 2 l/day, and WS_3: 1 l/day. The water deficit was carried out from the 17th to the 27th Eichhorn-Lorenz (E-L) stages. In order to emphasise details of the embryo development, a paraffin section method and technique was used. With the early water stress, there was no effect on the pollen viability and pollen germination ratios. The berry set ratio was reduced and the berry development were affected negatively by the early water stress. Between the 19th and the 27th E-L stages, an early water stress in 'Syrah' was noted, with decreases in the pre-dawn leaf water potential values below –0.8 MPa. The water deficit affected the berry growth and the development negatively.

Keywords: early water deficit; cv. 'Syrah'; berry development; grapevine; *Vitis vinifera* L.

Under Mediterranean climates, as a result of the global warming, precipitation irregularly falls throughout the year and the lack of water during the grapevine growth period is often observed. Grapevine growth and grape berry development are closely linked with soil water availability. Lengthy soil water deficits reduce the leaf area and photosynthesis (Hardie, Considine 1976), the vegetative growth, and the reproductive growth and the yield in the grapevines (Cifre et al. 2005). Different irrigation levels can cause physiological variances that affect the yield and berry composition (Esteban et al. 1999). Water deficits before veraison may have substantial effects on the berry development (Roby et al. 2004). Also, post-veraison water stress may reduce the berry size and compromise the berry composition (Ojeda et al. 2002; Bahar et al. 2011). As well, Antolin et al. (2008) speculated that the berry growth rate in the active cell division growth stage (stage I) in stressed vines can be faster than in non-stressed vines.

Mccarthy et al. (2002) reported that early water deficits reduce the berry cell division. Also, Williams and Matthews (1990) speculated that the late water deficits main effects could be the inhibition of the berry growth. The yield and quality in grapevines are not considerably reduced by moderate water deficits. In early stages of grape berry development, water availability may have an effect on the final berry maturity (Chaves et al. 2010). Also, Harris et al. (1968) reported that cell division and the production of the seed embryos takes place at the first rapid growth phase (i.e., stage I). The berry volume per vine, which sets the limit of

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the crop production, depends on the berry number and berry juice volume per berry (Conde et al. 2007). A basic method to modify the berry size is by controlling the soil water availability during the berry development (Coombe 1992; Bindon et al. 2008). It should not be forgotten that the berry set, and, thus, the yield, could improve by cultural practices such as irrigation (Collins, Dry 2009; Kamiloglu 2011). Our work was focused on determining the effects of early water stress levels on the berry development in 'Syrah'.

MATERIAL AND METHODS

Plant material and experimental layout. The experiment was conducted in 2008 in potted (70-liters) grapevines under vineyard conditions, using the SupAgro ECOTRON System in Montpellier (south of France). The vineyard was established in a north-south direction in 2001 with the 'Syrah' vines grafted on an SO4 rootstock. The vines were trained to bilateral cordons on a Lyre (Carbonneau et al. 2004) with approximately six spurs of 2–3 nodes each. The pot surface was protected from rainfall by using a black PE (polyethylene) material, and the growing medium was perlite and coarse sand with controlled drainage. Irrigation (drip) treatments were performed consistent with the methods according to Carbonneau (1998). The treatments were WS_0 (control): 4 l/day, WS_1: 3 l/day, WS_2: 2 l/day, and WS_3:1 l/day. Before starting the trial, the vines were thinned to about 30 clusters. The water deficit was carried out during the 17th to the 27th E-L stages (Eichhorn, Lorenz 1977). After that, all the plants were irrigated 6 l/day during the 27th to the 31st E-L stages.

The experimental set up was organised as a randomised complete block design (RCB) with three replications; every replication had two grapevines and four water stress levels.

Sampling. In the 17th E-L stage, pollen was collected from the flowers. Beside this, the flowers and berries were sampled from 24 vines at 3-day intervals (27th to 31st E-L). Fixation was undertaken in an FAA (formaldehyde 40%- acetic acid 45%- ethanol 95%) solution. The samples were collected from the vineyard and tested immediately. The pollen viability (%) was determined by acetocarmine staining, and the pollen germination ratio (%) was determined using agar in Petri dishes (agar 1% + sucrose 15%). The pollen viabilities and germinations were also viewed by a Leitz DMRB fluorescence microscope (Leica Microsystemes SAS, Nanterre Cedex, France). The berry set ratio (%) was also determined. The schedule of the paraffin method as described by Johansen (1940) was followed. The samples were embedded in Technovit® 9100 methyl methacrylate. Then, after using a Leica RM 2165 (Leica Microsystemes SAS, Nanterre Cedex, France), the microtome sections were cut by 5 μm and they were stained with 0.5% toluidine blue. An Olympus BX51 light microscope (Olympus France SAS, Rungis Cedex, France) was used to examine these slides.

Leaf water status. A Scholander pressure chamber was used to measure the pre-dawn leaf water potential (Ψ_{pd}) at 3-day intervals at 03:00 AM. Six fully-expanded leaves were measured per treatment (Scholander et al. 1965). The pre-dawn leaf water potential and grapevine water status thresholds were evaluated according to Carbonneau (1998) and Deloire et al. (2004) (Table 1).

Statistical data treatment. The analysis of variance (ANOVA) was performed using the MSTAT-C statistics program. The differences between the means of the treatments were compared using an LSD test for significant differences at a $P < 0.01$ level.

RESULTS AND DISCUSSION

Predawn leaf water potential (MPa)

The vine water status measured as Ψ_{pd} has previously been reported (BINDON et al. 2008). At the onset

Table 1. The pre-dawn leaf water potential and grapevine water status (according to Carbonneau 1998, Deloire et al. 2004)

Classes	Pre-dawn leaf water potential (Ψ_{pd}, MPa)	Level of water constraint or stress
0	0 MPa ≥ Ψ_{pd} ≥ –0.2 MPa	no water deficit
1	0.2 MPa ≥ Ψ_{pd} ≥ –0.4 MPa	mild to moderate water deficit
2	0.4 MPa ≥ Ψ_{pd} ≥ –0.6 MPa	moderate to high water deficit
3	0.6 MPa > Ψ_{pd}	high water deficit

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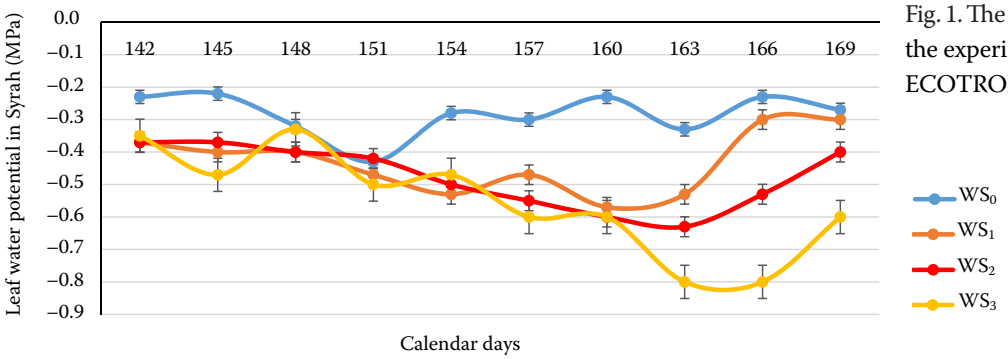

Fig. 1. The 'Syrah' Ψ_{pd} values during the experiment in the Montpellier ECOTRON System/2008 (MPa)

of the experiment, Ψ_{pd} was approximately between 0 and –0.30 MPa in all the treatments. The control (WS_0) was fully irrigated (4 l/day), so the Ψ_{pd} values were between –0.21 and –0.29 MPa. In the WS_1 Ψ_{pd}, the values ranged from –0.30 to –0.53 MPa; they were –0.33 to –0.63 MPa in the WS_2; and they were –0.80 MPa in the WS_3 (Table 1 and Fig. 1). When the plants were irrigated 163 days after the bud burst (6 l/day) there were increases in the Ψ_{pd} values across all the treatments.

Pollen viability and germination rate (%)

There was no difference in the pollen viability across the treatments (Table 2). The highest pollen viability value was measured in the WS_3 (95.76%) treatment. The lowest was measured at 93.71% in the WS_1 treatment. The others were: WS_2 (93.99%) and the control (94.21%), respectively. In Fig. 2, the dead pollen grains are indicated. On the other hand, there were differences in the pollen germination ratios between the treatments (Table 2, Fig. 3); the best one was WS_0 (41.91%).

Table 2. The pollen viability and germination rates of 'Syrah' in the Montpellier ECOTRON System/2008

	Pollen viability (%)	Pollen germination (%)	Berry set ratio (%)
WS_0	94.92	41.91^{a}	80.32^{a}
WS_1	93.71	37.79^{ab}	64.71^{b}
WS_2	93.99	32.50^{c}	70.85^{b}
WS_3	95.76	31.31^{bc}	54.81^{c}
Mean value	94.60	35.88	67.67

NS – not significant; Pollen germination LSD 5% = 8.27; Berry set ratio LSD 1% = 9.14

Berry set ratio (%)

The berry set ratios ranged between 54.81% and 80.32%, and there were differences according to the stress groups. Bessis (1993) stated that $a \geq 50\%$ or better berry set was normal and < 30% is poor. Baby et al. (2015) reported that the berry set ratio in cv. 'Syrah' is 67%. In this study, there was a normal berry set according to these guidelines (Table 2). Wenter et al. (2018) reported if Ψ_{pd} is ≈ –0.4MPa or greater, the fruit set is not affected the same as our study.

Berry (embryo) development

The berry development was examined cytologically at 10 sampling times across all the treatments. At the 1st sampling time (E-L 17; Fig. 4), the ovule was completely inverted 180° on its stalk. The micropyle was pointing towards the pedicel with the funiculus joined to the outer integument, forming a raphe

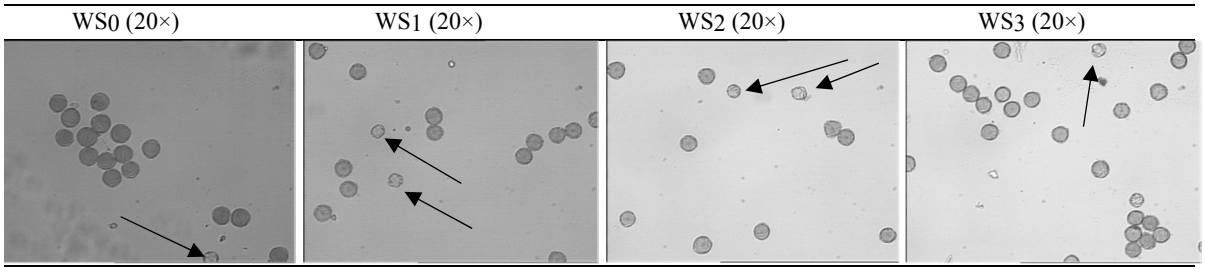

Fig. 2. The pollen viability micrographs in the 'Syrah' Montpellier ECOTRON System/2008 (the arrows point out the dead pollen grains)

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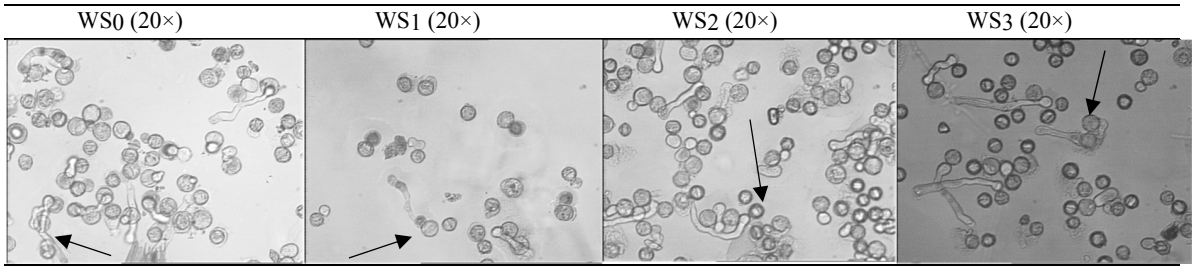

Fig. 3. The pollen germination micrographs in the 'Syrah' Montpellier ECOTRON System/2008 (the arrows point out the germinated pollen grains)

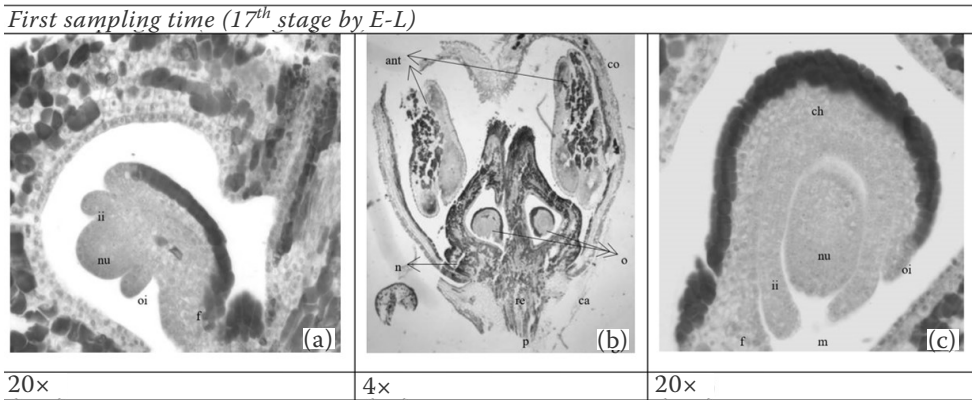

Fig. 4. The anatropous ovule, the conglomeration was formed in all the groups (a) the beginning of the ovule development, (b) the full flower with the two ovules, (c) the inner and outer integuments are well developed as well as the nucellus

nu – nucellus; oi – outer integument; ii – inner integument; f – funiculus; ca – calyx; co – corolla; n – nectar; re – receptacle; p – pedicel; o – ovule; m – micropyle; ch – chalaza

(anatropous) (Fig. 4). The inner and outer integuments and nucellus were well developed. There was no water stress at this stage (–0.23 to –0.37 MPa).

At the 2nd sampling time (E-L 19; Fig. 5a, b, d), the ovules were well developed. But, as seen in Fig. 5c, some defects occurred. The outer integument was thin. The inner integuments were not separated well and were thick. The micropyle aperture was almost closed, and the embryo sacs were surrounded by a nucellus. Also, the nucellus cells decreased numerically. A characteristic of the grapevine nucellus is the well-developed hypostase as well as the thick-walled tissue close to the chalaza (Pratt 1971; Mullins et al. 1992).

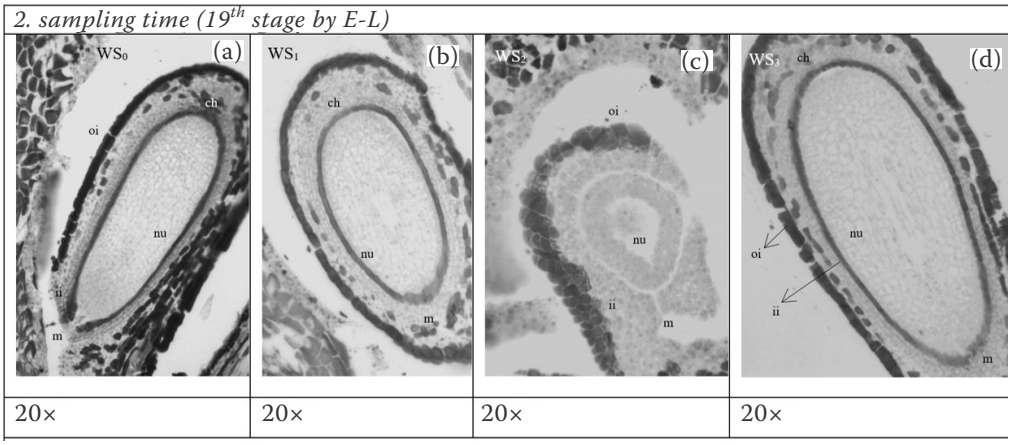

Fig. 5. The well-developed ovules belong to the stress groups (a, b and d), (c) Some malformations of the inner and outer integuments in the WS_2 (for abbr. see Fig. 4)

nu – nucellus; oi: outer integument; ii – inner integument; m – micropyle; ch – chalaza

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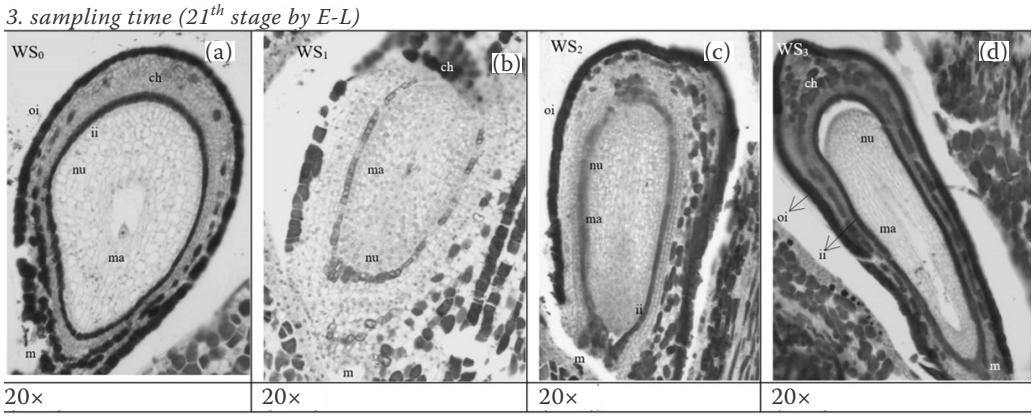

Fig. 6. The megaspores initially arranged in a linear tetrad in the WS1 and WS2 (b, c). Eight-nucleate embryo sacs start the formation, and an aperture seen in the centre of the embryo sac in the WS0 and WS3 (a, d)

nu – nucellus; oi – outer integument; ii – inner integument; m: micropyle; ch – chalaza; ma – megaspore mother cell

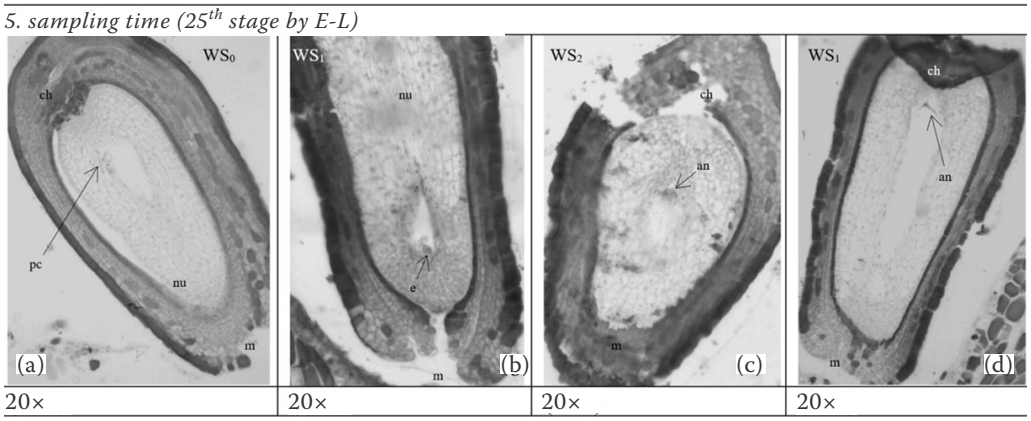

Fig. 7. The eight-nucleate embryo sac has developed, and the polar cells can be seen in the WS_0 (a), the micropyle aperture was open in the WS_1 (b), the antipodal cell is visible in the WS_2 (c) and the WS_1 (d)

pc – polar cell; e – egg cell; nu – nucellus; an – antipodal cell; m – micropyle; ch – chalaza

In the 3rd sampling period (E-L 21; Fig. 6), the embryo sac, a subepidermal cell of the nucellus, became an archeosporial cell and it divided periclinally to produce an outer primary parietal cell and an inner primary sporogenous cell. The primary sporogenous cells became the megaspore mother cells. The megaspores were arranged initially in a linear tetrad. The megaspore turned towards the chalaza and the remaining three megaspores degenerated. Then three mitotic divisions follow to produce an eight-nucleate embryo sac (Pratt 1971; Mullins et al. 1992). In this research there was a distinction seen towards the chalaza (Marasali 1992).

In the 5th sampling time (E-L 25; Fig. 7), the eight-nucleate embryo sacs had developed and they were of the polygonum type. There were three antipodal cells in the chalazal part of the embryo sac (Fig. 7c, d), with two polar cells centred in the embryo sac (Fig 7a) and there was an egg cell (Fig. 7b) and two synergid cells in the micropylar part of the embryo sac. The eight-celled embryo sac developments occurred normally.

In the 6th sampling time (E-L 25; Fig. 8), the eight-celled embryo sac development continued (Fig. 8a, b). In this stage, the flowers were open (Fig 8c). In the WS_3 nucellus cells, the eight-celled embryo sac degenerated and they coalesced towards the microphyl side. There were no nucellus cells seen on the chalazal side (Fig. 8c) (Ağaoğlu 1999). By the 7th sampling time (E-L 27; Fig. 9), the pollen tube ejaculated its content into one of the synergids prior to the fusion of the sperm nuclei into the egg and polar cells

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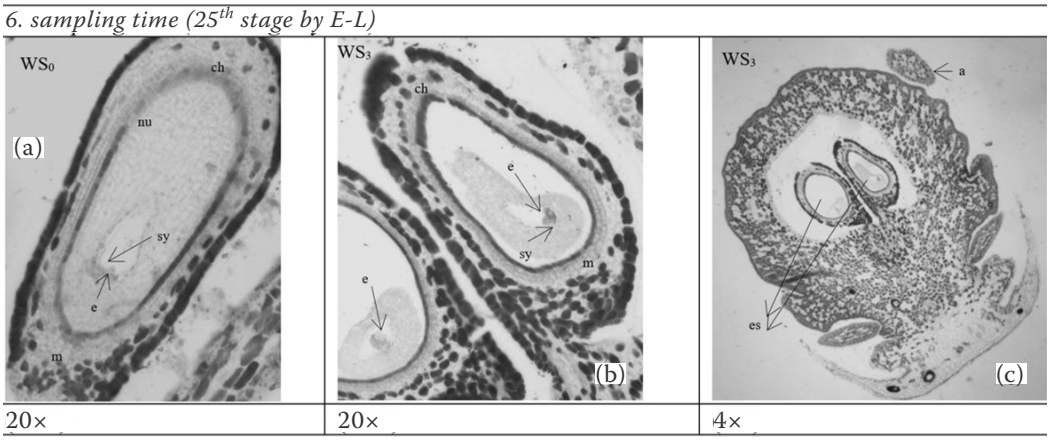

Fig. 8. The eight-nucleate embryo sac was developed, the synergids and egg cell can be seen in the WS_0 and the WS_3 (a, b), the flower was open in the WS_3 (c)

e – egg cell; nu – nucellus; sy – synergids; m – micropyle; ch – chalaza; a – anther; es – embryo sacs

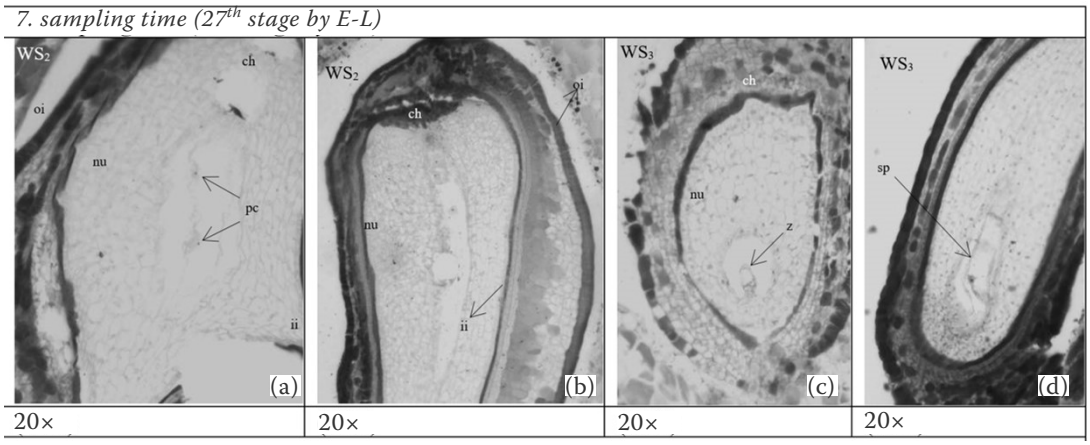

Fig. 9. The fertilisation has finished, in the WS_2 polar cells (a), in the WS_2 (b) the seed wall was formed. The sperm cells entered the embryo sac in the WS_3 (d). The zygote WS_3 (c)

nu – nucellus; oi – outer integument; ii – inner integument; ch – chalaza; pc – polar cell; sp – sperm cell; z – zygote

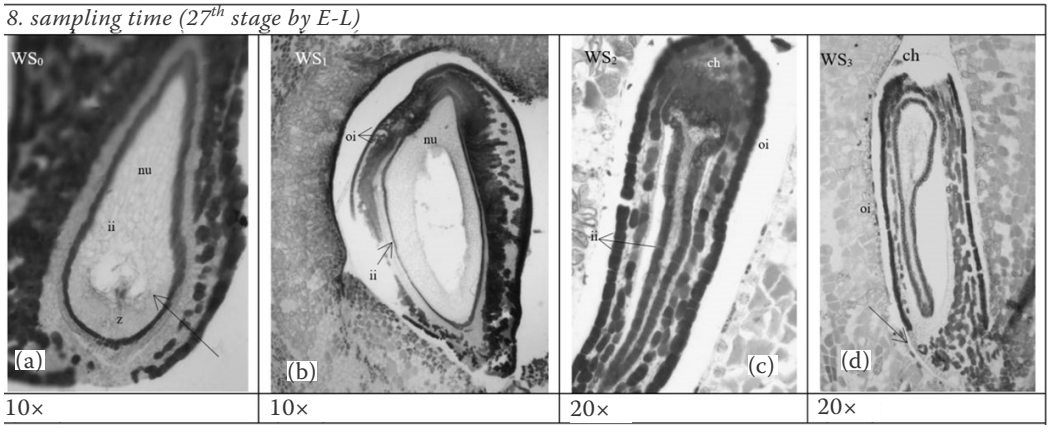

Fig. 10. The seed was formed and malformations were seen in the seed wall (a, b and c). There was a zygote in the WS_0 (a)

nu –nucellus; oi – outer integument; ii – inner integument; ch – chalaza; pc – polar cell; sp – sperm cell; z – zygote

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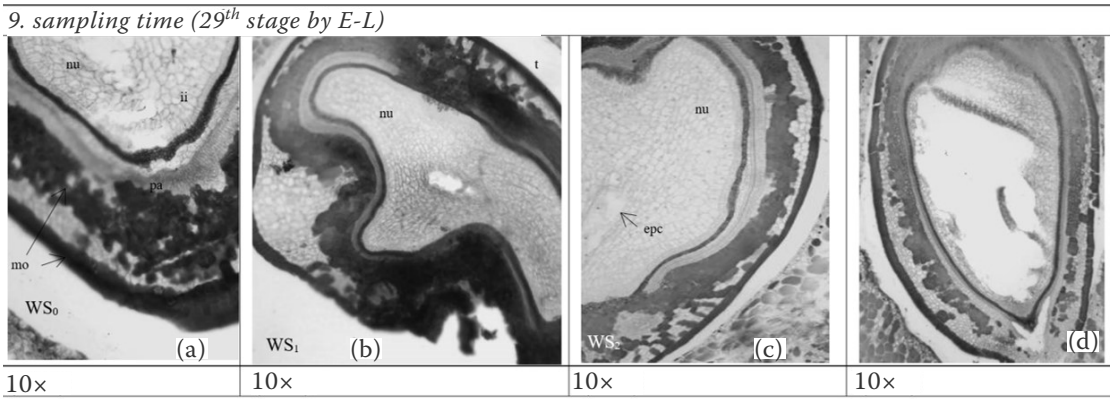

Fig. 11. The grape seed (a, b, c, d, e and f). The endosperm primer cell in the WS2 (d)

t – testa, ii – inner integument; oi –outer integument; pa – inner palisade layer; mo – middle and outer layers; nu – nucellus; epc – endosperm primer cell

(Fig 9d). At this time, the egg cell coalesced with the first generative cell (Fig 9c). Also, the polar cells were ready to coalesce with the second generative cell (Fig 9a). At this stage, the fertilisation was almost finished. Shortly after fertilisation, the rapid cell division in the funiculus, raphe, chalaza and integuments had started. At the micropyle, the outer integument was getting thicker and also elongated (Fig 9b).

In Fig. 10 (the 8th sampling; E-L 27), the seed coat had formed in all the stress groups. The outer integument changed to form a beak (Fig. 10b). But in the WS_2 and WS_3, the outer integument had not changed to form a beak. The outer integument became folded and a ridge was produced at the raphe towards the chalaza (Marasali 1992). On either side of the chalaza, two depressions or grooves normally formed, called fossettes (Mullins et al. 1992). This was the same as our study. However, some malformations were seen in the outer and inner integument layers according to the stress groups (Fig. 10a, c, d). Additionally, the endosperm was wrinkled and the zygote is not seen in the WS_1, WS_2 and WS_3.

At the 9th sampling stage (E-L 29; Fig. 11), after the berry set, a pincer-like growth was seen occurring, but not in the WS_3. The outer integument compressed the nucellus and the endosperm into a W-shape in the WS_0, WS_1 and WS_2. The testa contained an outer integument of three layers and the inner integument was surrounded by the nucellus (except for the WS_3). The outer integument's inner layer was a layer of palisade cells (WS_3), which were over the transfer cells layer within the inner integument.

At the 10th and final sampling (Fig. 12; E-L 31), depending on the stress levels, the occurrence of empty seeds happened. Mature grape seeds were formed in all the stress groups. The grape seed coat started to harden because of the lignification of the inner layers of the outer integument in the WS_0, WS_1 and WS_2 (Walker et al. 1999). This was the

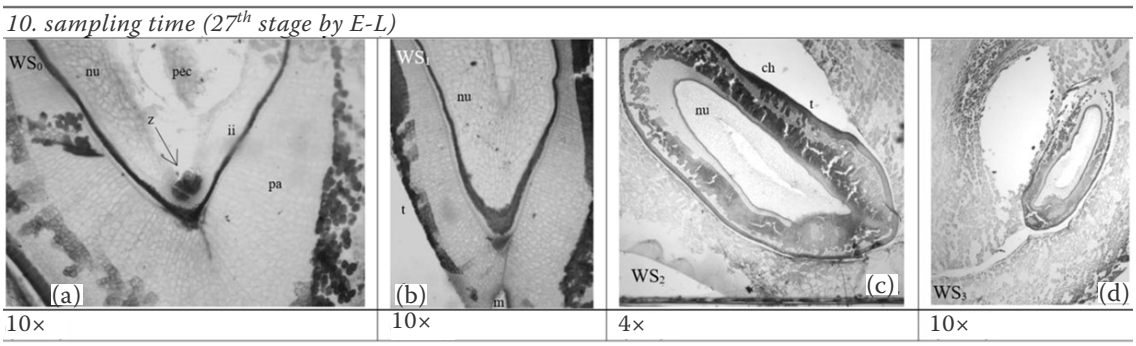

Fig. 12. The grape seed matured (a, b, c, and d). The zygote in the WS0 (a). The primer endosperm cells adhered to the inner integument layer

nu – nucellus; oi – outer integument layer; ii – inner integument layer; z – zygote; pec – primer endosperm cell; t – testa; pa – palisade layer; m – micropyle

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outer integuments inner layer (Marasali 1992). The zygote underwent an unequal division to produce a small terminal cell towards the chalaza and a large basal cell towards the micropyle. Mullins et al. (1992) reported that the pattern of the endosperm formation was classified as a helobial type.

CONCLUSION

Although the pollen viabilities were unchanged by the water stress (about 90%), there were differences found in the pollen germination ratios between the early water stress groups. The berry set ratios decreased with the increased water deficit, with the control at 80.3% and the severe water stress (WS_3) at 54.8%. The lowest Ψ_{pd} was in the WS_3, and, for that reason, some malformations in the berry seed coat and empty seeds were also formatted. Besides these findings, there was not any endosperm and zygote development seen in the WS_3.

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