International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 2 (2): 145-151, 2007
ISSN 1816-4897

The Effect of GA_3 and CCC on Ovule and Seed Development In Kabuğuyufka (*Vitis vinifera* L.)

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Abstract: Kabuğuyufka is one of the table grape varieties grown in Turkey. However, it shows both flower drop and shatter berry formation (millerandage). In this study, GA_3+CCC and CCC were applied for overcoming this problem of this grape variety and ovule development was also investigated. Plant Growth Regulator (PGR) application did not effect the anatomical structure of megasporogenesis and megagametogenesis. The abortion of embryo sac and ovule appeared after full bloom. Zygote degenerated two weeks after full bloom. Most of the control sample sections showed embryo and endosperm degeneration at about three weeks later than full bloom. Application of PGR's increased the number of developing ovule in the berry and slightly decreased the size of ovule in normal sized berries.

Key words: Grape, cultivar, PGR's, seed, berry formation

Introduction

Kabuğuyufka is one of the local table grape varieties in Erzincan in Turkey. However, it has severe flower and berry drops and different sized berries are formed on bunches known as "millerandage". Because of these problems, table grape quality and market value of Kabuğuyufka variety decreases. In this study, GA_3+CCC and CCC were applied to Kabuğuyufka grape cultivar for overcoming the shatter berry and different sized berry formation. CCC was used for both to increase berry set and to prevent flower and berry drop. In addition, a lower dose of GA_3 with CCC application was used for keeping the berry largeness. As usual, GA_3 has some physiological affects such as increasing the berry dimensions and decreasing the seed number.

Main purpose of the present study was to overcome both flower drop and shatter berry formation (millerandage). For this reason, the effect of PGR's (GA_3 and CCC) on ovule and seed development of Kabuğuyufka was investigated and the ovule and seed developmental phases were observed anatomically.

Plant Growth Regulators (PGR's) are commonly used for different aims in grape growing. Pollination and fertilization must take place for ovule development, seed formation and development in normal seeded grapes (Kassemeyer and Staudt, 1983). PGR's are also effective for fertilization and embryo development. Gibberellins which initiate cell enlargement and berry expansion and CCC which inhibits the plant growth are the first two PGR's (Ağaoğlu, 2002). The CCC inhibits the inner gibberellin synthesis (Aguero *et al*., 1995) and increases berry set and berry number but decreases the berry expansion (Coombe, 1967). The effect of GA_3 was tending to be dominant over CCC affection when GA_3 and CCC applied together. Therefore, the berry number decreases while their weight and volume increases with the increasing of GA_3 concentration (Considine and El-Zeftawi, 1971).

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The effect of GA_3 on fruit set varied with concentration, stage of development and whether the cv. was seeded, stenospermocarpic or parthenocarpic. With the seeded cvs GA_3 reduced the number of seeded berries but increased the number of seedless berries, the net effect usually being an increase. Applying CCC two weeks before anthesis generally enhanced set (Considine and Coombe, 1972).

In other prior study, with applications up to the time of full flowering, results in terms of yield and yield components were generally as good with GA_3 at 0.5 ppm + CCC at 50 ppm as with double these concentrations (El-Zeftawi and Weste, 1972).

CCC (100 ppm) + GA_3 (1 ppm), 4-CPA (20 ppm) + GA_3 (0.5 ppm) and GA_3 (10 ppm) had marked effects on yield and components of yield, but the response varied between sprays and years. In 1980 4-CPA + GA_3 produced more berries per bunch, heavier bunches and more fresh fruit per vine than both CCC+ GA_3 and GA_3, while in 1981, 4-CPA + GA_3 were superior to CCC + GA_3 (Cirami *et al*., 1983).

CCC (500 or 1000 ppm), applied one week before bloom, increased fruit set, the number of berries per bunch and bunch compactness but decreased fruit pedicel length and fruit weight. GA_3 applied 3 or 2 weeks before bloom plus CCC (500 ppm) applied one week before bloom, increased fruit set, pedicel length, the number of berries per bunch and bunch weight but had no effect on bunch compactness (Hifny *et al*., 1980).

Flower clusters of grape cv. Kyoho were dipped before bloom in GA_3 (1 ppm), IAA (100 ppm), BA (100 ppm), ABA (100 or 200 ppm), CCC [chlormequat] (200 ppm) or SADH [daminozide] (500, 2500 or 5000 ppm). The growth promotors tested had no significant effect on percentage set of seeded berries (Komatsu and Nakagawa, 1991).

Materials and Methods

The present study was carried out at the vineyards of the Erzincan Province in Turkey. The Gibberellic acid (GA_3) and CCC were applied to overcome both flower drop and shatter berry formation (millerandage) in Kabuğuyufka grape variety at the pre-and-after full bloom (15 days before full-bloom and 5 days after full-bloom) stages. Single or combined treatments with GA_3 and CCC were used at concentrations of 5 and 100 ppm (Table 1). There were 4 replicates and each replicate include 4 grapevines. GA_3 and CCC were sprayed through out the vine canopy. The Kabuğuyufka grape variety bloomed in the third week while full bloom occurred in the last week of the June.

Both flower and berry samples were taken at 5 days interval between 10^{th} day before the beginning of full bloom and 20^{th} day after full bloom. Flower and berry samples were fixed in FAA (70% ethanol 85 ml, neutral formalin 5 ml and glacial acetic 5 ml) solution. Sixteen samples were taken randomly at each time. Both normal and smaller berries at the cluster were fixed separately and examined. Longitudinal and transversal cross-serial-sections were performed at 10 μm with microtome, stained with safranine and fast green and mounted in Canada balsam (Odabaş, 1976). The number of ovules in the berries was counted through transversal sections of the ovules. In the longitudinal sections, the ovule length and width were measured with an ocular micrometer.

Table 1: The GA_3 and CCC applications and their application times

Applications	(ppm)	Application time
I	GA_3 + CCC* (5)+(100)	15 days before full bloom
II	GA_3 + CCC** (5)+(100)	15 days before full bloom and 5 days after full bloom
III	CCC* (100)	15 days before full bloom
IV	CCC** (100)	15 days before full bloom and 5 days after full bloom
V	Control	Distilled water were sprayed both 15 days before and 5 days after full bloom

Results

Effect of PGR's on the Anatomical Development of Ovule

It was found that there were 4-8 ovules in each flower ovary with two ovules in a carpel before bloom (the fully formed grape flower stage) (Fig. 1a). Most of these ovules failed to develop in further periods and some of them could complete their development. In this period, one of the nucellus cell as megaspore mother cell formed four megaspores with meiosis (Fig. 1b). One of these four megaspore cells developed and the others degenerated. The developed megaspore was divided tree times with mitosis and formed embryo sac having 8 nucleate. Investigations showed that the application of PGR's had no effect on the anatomical structure of the ovules compared to the control samples.

The ovule length and width were 0.623 and 0.315 mm in the stage of full bloom. Polar nuclei fused and resulted in diploid secondary nucleus formation forwarding to micropyle (Fig. 1c-f). One of the generative nucleate of pollen incorporated with egg cell and formed zygote, the other incorporated with seconded cell and formed primary endosperm nucleus (Fig. 2a). The first division in the primary endosperm nucleus appeared a week after full bloom and one of the endosperm nuclei was seen in the middle of embryo sac while the other took place in chalazal tip. The inner integument retained thick but outer one showed multiple layered structure. The ovule length and width reached to 1.009 and 0.574 mm, respectively.

It was seen that there were a rapid dilate in the ovules at about two weeks from the full bloom. The endosperm nucleus was determined in all normal sized ovules but zygote could not been seed in some samples. It was determined that the ovules without both zygote and endosperm were smaller than the others. However some samples showed zygote vacuolization known as zygote degeneration (Fig. 2b).

It was determined that 17-18 days elapsed from fertilization to the first division of the zygote (Fig. 2c, d). The endosperm developed rapidly in a temporary period before this stage and nearly 15-50 endosperm nuclei were counted in the embryo sac. It was found that there were just 7-8 endosperm nuclei in the under developed ovules than normal ones. The embryo and endosperm degeneration were determined in the most of the control samples about three weeks after full bloom. While some samples had only endosperm, the others showed neither endosperm nor embryo development. Nucellus tissue spread on a wide area in the ovules. And sclarenkimatic cells formed at outer integument tissue. This tissue forms the hard parts (testa) of the seeds (Fig. 2e).

Abortion of the ovules and embryo sac appeared a week after full bloom and continued during the further periods. In the aborted ovules, the embryo sac and nucellus with inner integument cells wrinkled and separated from outer integument of the ovule (Fig. 2f). It was determined that the abortive ovule length did not exceed 0.639 mm.

Effect of PGR's on the Development of Ovule into the Berry

Data obtained from PGR's application on the ovule number and their dimensions of Kabuğuyufka grape cultivar were presented at the Table 2. According to the results, PGR's application increased the developing ovule number into berry. The average ovule number in the control samples were just as 1.00-2.00 while PGR's applications raise to 3.50, approximately. The highest ovule number into a berry was obtained from GA+CCC* application. However, the ovule lengths were found to be slightly smaller in PGR's applications than control ones in the normal size berries.

In the present study, it is obviously clear that the number of ovule in the normal sized berries is much more than the smaller ones at the samples taken 15^{th} and 20^{th} days after full bloom.

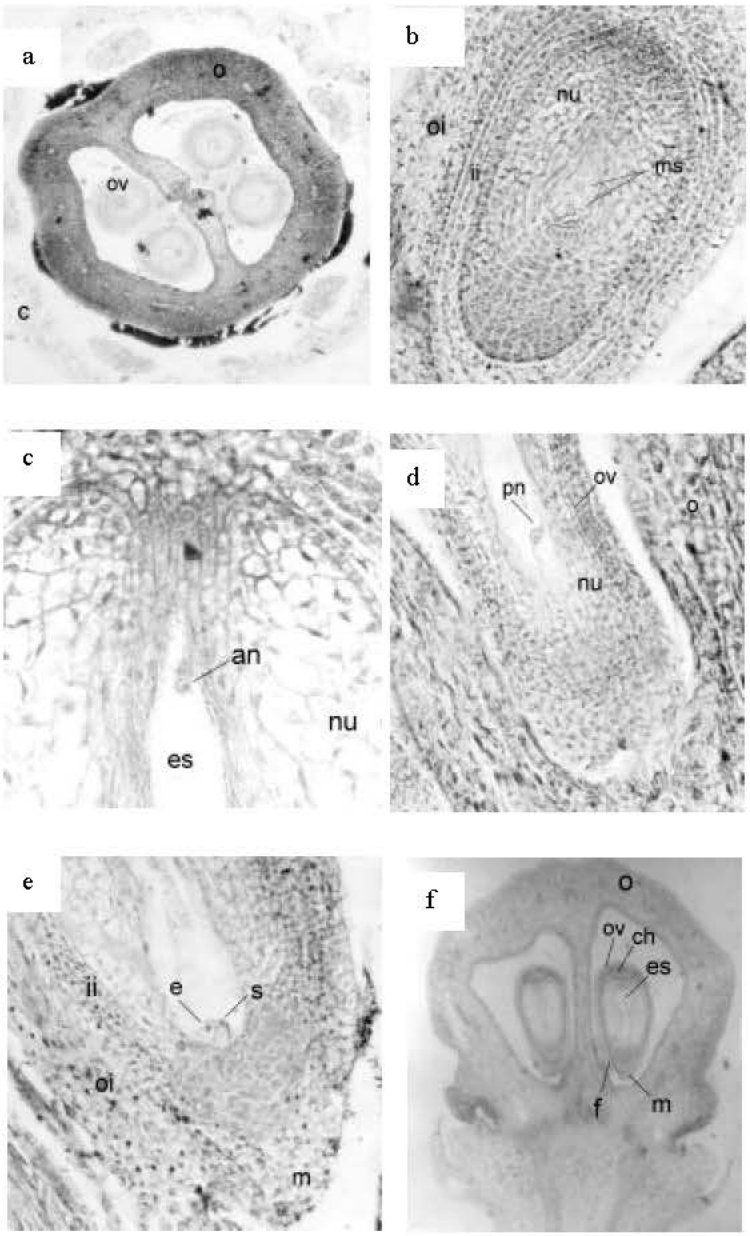

Fig. 1: Ovule development stages of Kabuguyufka grape cultivar before fertilization. (a) ovary with two carpels before dehiscence of caliptrae, 40X, (b) megaspores at egasporogenesis, 200X, (c) antipodals are visible, 400X, (d) fused polar nukleus, 200X, (f) embryo sac with egg cell and synergids, 200X, (f) longitudinal section of the mature flower, 40X. c: corolla, o: ovary, ov: ovule, nu: nucellus, ms: megaspore, oi: outher integument, ii: inner integument, an: antipodals, es: embryo sac, pn: polar nucleus, e: egg cell, s: synergids, m: micropyle, f: funiculus, ch: chalaza

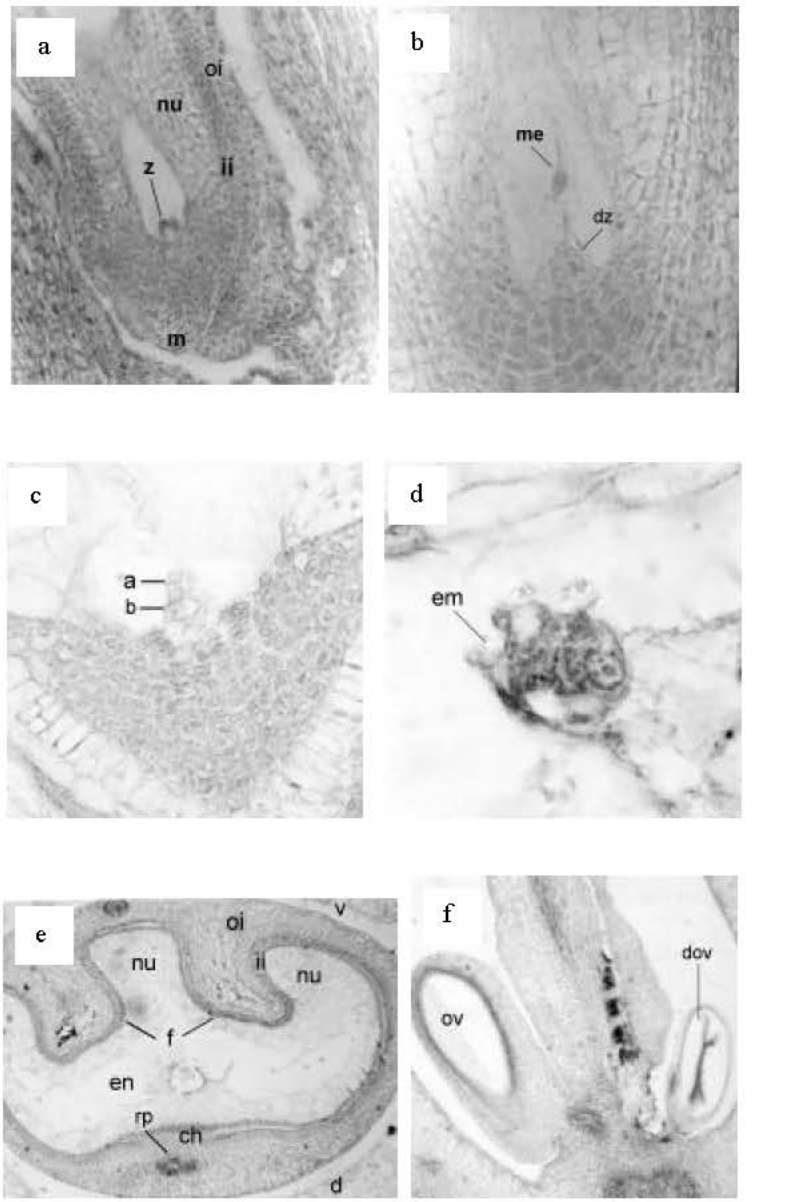

Fig. 2: Ovule development stages of Kabuguyufka grape cultivar after fertilization. (a) fertilized ovule and zygote, 200X; (b) degenerated zygote, 400X; (c) proembryo, 400X; (d) poly celled embryo, 1000X; (e) transversal section of ovule at poly celled embryo stage, 40X; (f) degenerated ovule, 40X. z: zygote, nu: nucellus, oi: outher integument, ii: inner integument, m: micropyle, me: micropilar endosperm nucleus, dz: degenerated zygote, a: apical cell, b: basal cell, em: embryo, dov: degenerated ovule, ov: ovule, f: fossettes, d: dorsal side, v: ventral side, ch: chalaza, rp: raphe, en: endosperm

Table 2: The effects of GA_3 and CCC application on the number and dimensions of ovules at Kabuğuyufka grape cultivar

Sampling times (Days after full bloom)	Berry size	Ovule	Applications (I) GA_3 + CCC*	(II) GA_3 + CCC**	(III) CCC*	(IV) CCC**	(V) Control	LSD
		Number	1.50ab	1.00b	1.00b	2.00a	1.00b[1]	0.6329[2]
	Normal	Length (mm)	2.576	2.445	2.520	2.240	2.640	NS
10		Width (mm)	0.893b	0.985b	1.148a	0.980b	1.008b	0.11912[2]
		Number	3.50a	1.50b	1.50b	1.50b	1.00b	0.6260[3]
	Small	Length (mm)	1.036b	1.204b	0.924b	1.568a	1.176b	0.3206[2]
		Width (mm)	0.263c	0.840ab	1.14a	0.952a	0.560bc	0.3001[3]
		Number	2.00	3.00	2.00	2.00	2.00	NS
	Normal	Length (mm)	2.940b	3.090b	3.976a	4.396a	4.564a	0.7797[3]
15		Width (mm)	2.016	2.100	2.100	2.408	2.408	NS
		Number	3.00a	1.50b	1.50b	1.00b	2.00ab	1.0730[2]
	Small	Length (mm)	2.520c	4.480a	4.312a	4.284a	3.640b	0.3970[3]
		Width (mm)	2.520a	2.744a	1.736b	2.268a	2.604a	0.4910[2]
		Number	3.00a	2.50ab	1.50bc	2.50ab	1.00c	0.9686[3]
	Normal	Length (mm)	4.424b	3.780b	4.312b	4.340b	5.768a	1.0160[2]
		Width (mm)	3.192ab	2.520c	2.604bc	3.052abc	3.220a	0.5747[3]
20		Number	3.00a	1.50b	1.00b	1.00b	1.00b	1.225[3]
	Small	Length (mm)	4.256b	4.060b	4.816a	4.228b	4.592ab	0.5087[2]
		Width (mm)	2.016bc	1.792c	2.604ab	1.792c	2.800a	0.6159[2]

*Application once, **Application twice, [1]Mean followed by the same letter on the lines is not significantly different
[2]$p<0.05$, [3]$p<0.01$, NS: Non Significant

Discussion

The cross section, taken after a week or later from full bloom, showed that aborted ovules had aborted-embryo sac and nucellus cells. The aborted ovules' length did not exceed to the average ovule length (0.6 mm) determined at the full bloom. This proved that there were not any development of ovules after full bloom According to this evidence, the abortion of embryo sac in some ovules happened due to insufficient pollination and fertilization. The same findings were also stated by Barritt (1970) and Ebadi *et al*. (1996).

In the present study, some samples taken after two weeks from bloom, zygote could not appear even if endosperm developed, however zygote vacuolization defined as zygote degeneration appeared in some samples. The endosperm nucleuses were determined in all normal sized ovules. It was determined that the ovules without zygote and endosperm were smaller than the others. The previous studies also indicated the same results as our findings (Barritt, 1970; Marasalı, 1992).

Embryo and endosperm degenerations were determined after about three weeks from full bloom. This degeneration was more frequent in untreated-samples. But, the ovules form a normal seed resulted in empty seed structure. As Ebadi *et al*. (1996), Peynaud and Riberau-Gayon (1970) and Ağaoğlu (2002) reported that, the berries having normal sized seeds are bigger than the berries having undeveloped embryo (empty seeds). And also, there are positive relations between seed number and fresh berry volume, dry matter content and accumulation of photosynthetic products.

Aguero *et al*. (1995) and Çelik (1998) stated that the CCC had a preventative effect on gibberellin synthesis resulting in ovule abortion. They also indicated that it increased the berry set and ovule number but decreased the berry size. In this study, the negative effects of CCC on seeded grape cultivar were hindered by applying the lowest GA_3 doses. We found that, the application of PGR's increased the number of developed-ovules and slightly decreased the ovule dimension in normal-sized-berries.

As a result, PGR's application decreased the degeneration and abortion in ovule development stages. Furthermore PGR's application increased ovule number per berry. Thus berry drop and millerandage could be suppressed by applying the PGR's on Kabuğuyufka grape cultivar. And uniform bunches could be obtained.

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