EFFECT OF EXOGENOUS GIBBERELLIC ACID AND PRESENCE OR ABSENCE OF TESTA ON THE EX VITRO SEEDLING GROWTH OF BAY LAUREL (*LAURUS NOBILIS* L.)

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Abstract. Seed germination, seedling growth and viability are the initial, critical steps and they are affected by various exogenous and endogenous factors including seed testa and plant growth regulators. This study investigated the effects of testa (seeds with or without testa) and gibberellic acid (GA_3; 0, 100, 500, 1000, 2000, 3000 and 4000 ppm) on seed germination, seedling growth parameters and seedling viability rate in bay laurel (*Laurus nobilis* L.). It has been definitely demonstrated that the seed testa extends seedling formation process at the final germination by nearly two times via delaying effects on the first germination time. For seed-with testa, all GA_3 concentrations were found to be similarly successful (between 83.3 and 88.3%) compared to control (68.3%) but in seed-without testa, high GA_3 concentrations (55.0% in 2000 ppm, 56.7% in 3000 ppm and 55.0% in 4000 ppm) showed greater negative effect on final germination than lower doses (80% in 100 ppm, 81.7% in 500 and 1000 ppm) and even control (63.3%). GA_3 concentrations showed no differences in most seedling parameters excluding root number in seeds with testa and maximum root length in seeds without testa. Despite the delaying effect of testa, seedling and viability capacity were higher than those of seeds without testa in high GA_3 concentrations. On the other hand, in control and lower doses of GA_3 (100, 200 and 500 ppm) gave similar results of these two parameters. All data confirmed that *Laurus nobilis* L. seed testa have effects on seed germination delaying but found more resistant to seedling capacity (83.09%) and seedling viability (94.39%) in average after acclimatization in which seed-without testa gave lower results with 67.63%; 78.24% respectively in the *ex vitro* studies.
Keywords: *Lauraceae, seed coat, germination, gibberellins, abiotic stress, plant physiology*

Introduction

Seed germination and seedling success is a vitally important factor not only for human nutrition, but for animal feeding, pharmaceutical and other livelihood occupations as well, it is also an important factor in protecting plants from environmental damage and maintaining generations especially plants which are substantially collected from nature mostly without setting a plantation as *Laurus nobilis* L.

In some conditions seed plants may contain some types of dormancy. There is quite a bit of diversity in dormancy at physiological, morphological and anatomical levels. Physical dormancy is one of the types and is caused by one or more water-impermeable layers of palisade cells in the seed or fruit coat. Mechanical or chemical scarification will promote germination in seeds with non-deep physiological dormancy (Baskin et al., 2000; Baskin and Baskin, 2004). Testa is maternal tissue, it is inherited maternally and in typical angiosperm seeds the embryo is surrounded by two covering layers: the endosperm and the testa (seed coat) (Finch-Savage and Leubner-Metzger, 2006). At the same time some seeds in the plant kingdom are recalcitrant and do not maintain high viability in storage, undergo little or no maturation drying and remain desiccation

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sensitive both during development and after they are shed (Berjak and Pammenter, 2002). Recalcitrant seeds typically have high water content, and well-developed embryos (Bannister et al., 1996).

Although there are numerous studies about dormancy types, dormancy breaking and germination requirements for seed (Baskin and Baskin, 2004; Rowarth et al., 2007; Tang et al., 2019; Sharma et al., 2020; Koutouan-Kontchoi et al., 2020) and numerous studies about seed behavior as orthodox, recalcitrant and intermediate (Berjak and Pammenter, 2002; Jaganathan et al., 2019; Viana et al., 2020; Azarkovich, 2020; Bharuth et al., 2020), there are limited studies focused on seed coat effects on seed germination of woody plants (Sari et al., 2006; Li et al., 2012; Gendreau and Corbineau, 2009).

Gibberellins are a group of plant hormones stimulating growth and development in nearly all steps including stem and internode elongation, regulation of flowering, male and female fertility besides germination. Seed germination is a complex process, controlled by both physical and internal regulating factors. Gibberellins are required for breaking seed dormancy (Gupta and Chakrabarty, 2013). There are limited studies about breaking dormancy in seeds with the aim of external usage of gibberellic acid in woody plants (Rehman and Park, 2000; El-Dengawi, 2005; Çetinbaş and Koyuncu, 2006; Fang et al., 2006) than monocots.

The model plant *Laurus nobilis* L. of the Lauraceae family is an evergreen, perennial, dioecious plant native in Turkey, grown as high-value spice crop, ornamental, importance of ecological and economical aims. Active chemicals from different plant parts are mostly obtained from traditionally grown or naturally existing plant. The high economic value of the species in Lauraceae has caused these to be destroyed in the natural habitats over years according to personal long-term observations.

The evergreen plant has one-seeded drupa fruits, that are oval shaped, and become green to glossy black (Aytürk and Ünal, 2013). There has been an increasing interest to the plant because of its biologically active substances from the under threatened plant. The plant has antimicrobial (Fernández et al., 2019; Nafis et al., 2020), antioxidant (Rincón et al., 2019; Hussein et al., 2019), pharmaceutic (Duletíc-Laušević et al., 2019; Riabov et al., 2020; Chbili et al., 2020) and plant protective (Ebrahimi et al., 2013; Fidan et al., 2019) chemical composition. The odor is caused by volatile chemicals and mostly fixed or essential oils that are found in different ratio in different plant parts as flower, leaf, bark, berry, seed etc. (Kilic et al., 2004; Yılmaz and Deniz, 2018).

Because of importance of *Laurus nobilis* L. some studies about its seed dormancy and storage behavior (Takos, 2001; Takos and Efthimiou, 2003; Sari et al., 2006; Konstantinidou et al., 2008; Ertekin and Çorbacı, 2018) have been carried out. Takos and Efthimiou (2003) emphasized that laboratory tests are more successful than field sowing because of low temperature in autumn, in that time *L. nobilis* fruit is getting mature. In another study on *L. nobilis*, Takos (2001) it was shown that removing pericarp and cold stratification lead to a big success in germination compared to intact pericarp. Ertekin and Çorbacı (2018) used cold stratification or gibberellic acid and polystimulins in combination with cold stratification and they found out that all treatments are higher in germination criterion than control and higher in most of plant growth criterions. Sari et al. (2006) also found that the highest germination rate was observed when seeds were sowed after completely removing seed coat. The results of the one of before study showed that the laurel seeds are recalcitrant and seed coat extended the germination time so the seedlings have to be grown as soon as possible (Cavusoglu et al., 2014).

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The purpose of the study was to investigate the effects of presence or completely removed seed coat and different doses of exogenous gibberellic acid (GA_3) on duration of seedling growth and plant growth criterions of *Laurus nobilis* L. which has increasing medicinal, aromatic and environmental importance.

Materials and methods

A pot experiment was conducted between 2017-2018 and 2018-2019 (December to May) at the Faculty of Agriculture and Natural Sciences, Kocaeli University, Turkey. The bay laurel fruits were collected from only one full grown female plant (*Fig. 1a, b*) for both years in November 12, at an orchard near private property in Kocaeli city, located at 57 m a.s.l. with coordinates 40°.71.8312 N and 29°.99.1608 E. In the first year of the study, a hundred randomized fruits were measured on the day of collection (*Fig. 1c*) and in average one fruit was found 1.12 g in weight, 11.2 mm in diameter and 14.5 mm in length. All fruits were left in the refrigerator for 1 months at 8 °C prior to usage.

Figure 1. *Laurus nobilis L.; (a) initial female plant, (b) a fruity branch sample of the used plant, (c) a fruit cluster sample of the used plant, (d) fruit and used seed samples of the plant – seed with pericarp (fruit), seed-with testa, seed-without testa from left to right*

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At the treatment day narrow and skinny fruit pericarp of half of fruits were removed to obtain seed-with testa and on the other side pericarp and testa of the other half of seeds were removed to obtain seed-without testa (naked seed) (*Fig. 1d*). All this step performed manually with paper towel without the help of water etc. At this step a hundred seeds with testa and without testa were measured; one was found 0.72505 g to 0.682025 g in weight, 9.7 mm to 9.5 mm in diameter, and 11.9 mm to 11.3 mm in length respectively. GA_3 concentrations were prepared the same day as 100, 500, 1000, 2000, 3000 and 4000 ppm besides 0 (Control), and prepared seeds were left in the prepared GA_3 solutions for 24 h in glass beaker under laboratory conditions. Control seeds were left in the same amount of water for the same duration (*Fig. 2a*). The treated seeds were placed and sowed without washing 1 cm deep from surface in trays filled with 0.5 dm^3 base substrate peat: perlite (1:1, w:w) without added nutrients (*Fig. 2b*). The trays were watered to field capacity and placed in the room (temperature between 16 and 23 °C; relative humidity between 57 and 75%) of the laboratory for 6 weeks for the naked-seeds and 12 weeks for the seeds-with testa. Then rooted and shooted seedlings were acclimatized to the greenhouse (temperature between 25 and 39 °C), where they remained for 8 weeks (*Fig. 2c, d*). The trial lasted a total of 14 weeks for naked-seeds and 20 weeks for seeds-with testa in both years. This was because the last germination needed a longer time in seeds with testa.

Figure 2. *Seed germination and seedling steps of Laurus nobilis L. plant; (a) GA_3 treatment to seeds with testa and seeds without testa separately, (b) Initial germination as shoot reached at least 1 cm on substrate surface, (c) A sample of germination and seedling steps, (d) Acclimatized seedling of Laurus nobilis L. in glasshouse*

The seedling growth (at least 1 cm primer shoot on the surface of substrate) was recorded once a week and at the end of both tests (testa and GA_3 effects); root

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number, maximum root length, average root length, shoot length per seedling and seedling capacity were calculated for seeds with testa or seeds without testa separately at the end of the seedling growth under ex vitro laboratory condition because of taking different duration. End data of seedling and viability capacity after 8 weeks acclimatization were also calculated comparatively. All treatments were tested in two years with 3 replications and each replication consisted of 20 seeds. In total 840 seeds (420 seeds-with testa and 420 seeds-without testa) were used in each year. Results were analyzed by ANOVA and Duncan Multiple Range Tests ($p<0.05$) were done to determine differences among the data of the treatments in average of the two years.

Results and discussion

For the treatment with or without testa in bay laurel, the highly significant differences among them were recorded for first seedling, last seedling and average seedling week. All parameters show earliness in naked seed than in seeds with testa (*Tables 1* and *2; Figs. 3* and *4*). In average, seeds with testa began to turn seedling on 5.43^{rd} week first and on 10.87^{th} week last on the other hand seeds without testa began to turn seedling on 2.41^{st} week first and on 4.89^{th} last (*Table 3*).

Table 1. *Seedling capacity of seed with testa treated with different concentration of GA_3 of Laurus nobilis L. along 12 weeks*

GA_3 concentration	**1.week***	**2.week***	**3.week***	**4.week***	**5.week****	**6.week****
Control	0	0	0	0	1.7 ab	11.7
100 ppm	0	0	0	0	1.7 ab	13.3
500 ppm	0	0	0	1.7	1.7 ab	6.7
1000 ppm	0	0	0	0	5.0 ab	13.3
2000 ppm	0	0	0	0	5.0 ab	20.0
3000 ppm	0	0	0	0	6.7 a	16.7
4000 ppm	0	0	0	0	0.0 b	13.3
Average in week	**0**	**0**	**0**	**0.24**	**3.11**	**13.57**
	7.week**	**8.week****	**9.week****	**10.week****	**11.week****	**12.week****
Control	18.3 b	36.7 d	53.3 b	61.7 b	65.0 b	68.3 b
100 ppm	35.0 a	53.3 bc	66.7 ab	81.7 a	86.7 a	88.3 a
500 ppm	30.0 ab	51.7 cd	71.7 ab	76.7 ab	78.3 a	83.3 a
1000 ppm	43.3 a	68.3 ab	75.0 a	83.3 a	83.3 a	86.7 a
2000 ppm	40.0 a	58.3 abc	65.0 ab	73.3 ab	81.7 a	83.3 a
3000 ppm	38.3 a	71.7 a	78.3 a	80.0 ab	85.0 a	85.0 a
4000 ppm	45.0 a	70.0 a	80.0 a	85.0 a	86.7 a	86.7 a
Average in week	**40.80**	**58.57**	**70.00**	**77.39**	**80.96**	**83.09**

*N.S., No significant difference in GA_3 concentrations in seedling capacity of seeds-with testa
**Lower-case letters denote significant differences at the $p<0.05$ level in GA_3 concentrations in seedling capacity of seeds with testa

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Table 2. *Seedling capacity of seeds without testa treated with different concentration of GA_3 of Laurus nobilis L. along 6 weeks*

GA_3 concentration	1.week*	2.week**	3.week**	4.week**	5.week**	6.week**
Control	0	6.7 ab	40.0 b	56.7 ab	61.7 abcd	63.3 ab
100 ppm	0	10.0 a	46.7 ab	63.3 ab	73.3 abc	80.0 a
500 ppm	0	3.3 ab	61.7 ab	73.3 a	81.7 a	81.7 a
1000 ppm	0	3.3 ab	70.0 a	73.3 a	75.0 ab	81.7 a
2000 ppm	0	0.0 b	48.3 ab	48.3 b	51.7 d	55.0 b
3000 ppm	0	1.7 b	43.3 ab	55.0 ab	56.7 abc	56.7 b
4000 ppm	0	3.3 ab	50.0 ab	53.3 ab	53.3 cd	55.0 b
Average in week	**0**	**4.04**	**51.43**	**60.46**	**64.77**	**67.63**

*N.S., No significant difference in GA_3 concentrations in seedling capacity of seeds-without testa

**Lower-case letters denote significant differences at the $p<0.05$ level in GA_3 concentrations in seedling capacity of seeds-without testa

Table 3. *First week, last week and average week of seedling capacity of seeds with or without testa treated with different concentrations of GA_3 of Laurus nobilis L.*

GA_3 concentration	First seedling Week***		Last seedling Week***		Average seedling Week***	
	Seed with testa*	Seed without testa**	Seed with testa**	Seed without testa*	Seed with testa**	Seed without testa*
Control	5.7 A	2.3 ab B	11.7 bc A	5.0 B	6.0 b A	2.7 B
100 ppm	5.7 A	2.0 a B	10.7 abc A	5.3 B	5.0 ab A	3.3 B
500 ppm	5.3 A	2.3 ab B	12.0 c A	5.0 B	6.7 b A	2.7 B
1000 ppm	5.0 A	2.3 ab B	10.7 abc A	5.3 B	5.7 b A	3.0 B
2000 ppm	5.3 A	3.0 b B	11.3 abc A	5.0 B	6.0 b A	3.3 B
3000 ppm	5.0 A	2.7 ab B	10.0 ab A	4.3 B	5.0 ab A	1.6 B
4000 ppm	6.0 A	2.3 ab B	9.7 a A	4.3 B	3.7 a A	1.7 B
Average	**5.43**	**2.41**	**10.87**	**4.89**	**5.44**	**2.61**

*N.S., No significant difference in GA_3 concentrations on first seedling week in seeds with testa, last seedling week in seeds without testa and average seedling week in seeds without testa

**Lower-case letters denote significant differences at the $p<0.05$ level in GA3 concentrations on first seedling week in seeds without testa, last seedling week in seeds with testa and average seedling week in seeds with testa

***Capital letters denote significant differences at the $p<0.05$ level in same GA3 concentrations between seeds with testa and seeds without testa on first seedling, last seedling and average seedling week

According to the results of a study (Sari et al., 2006) supporting this; when seed coat completely removed, time to 50% of final germination took 2 weeks and total germination percentage found 85%. The findings statistically found shorter time consuming than control which is seed without only pericarp (33%) and time to 50% final germination took 7 weeks. In our previous study on mother plant type, seed age and seed coat effect on bay laurel germination (Cavusoglu et al., 2014), we found that first and last germination time showed earliness in naked-seed than seed with coat in both of tested female plant types. In another study on dormancy-breaking and

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germination requirements for seed of *Sorbus alnifolia* (Tang et al., 2019) after removing of pericarp; seeds which are scarified seed coat germination reached 8% when germination of intact seed was 0%. The study on complexities in identifying seed storage behavior of hard seed-coated Lauraceae species (Jaganathan et al., 2019) also emphasized that the lots of reasons of germination barrier of Lauraceae species include seed coat. Baskin and Baskin (2004) emphasized that in most cases, inhibition of protrusion of radicle require longer period to germinate and this results in physiological dormancy which essentially is inability of seeds to germinate despite seed coat is permeable to water. In a detailed study on seed dormancy and dormancy-breaking conditions of 12 West African woody species (Koutouan-Kontchoi et al., 2020), it was emphasized that if scarified coat seeds imbibed more water than intact seeds, then the seeds have an impermeable seed-coat hence physical dormancy. If this is not the case, it means that the seeds are non-dormant or could have another dormancy type and they found two distinct groups of used species in their study.

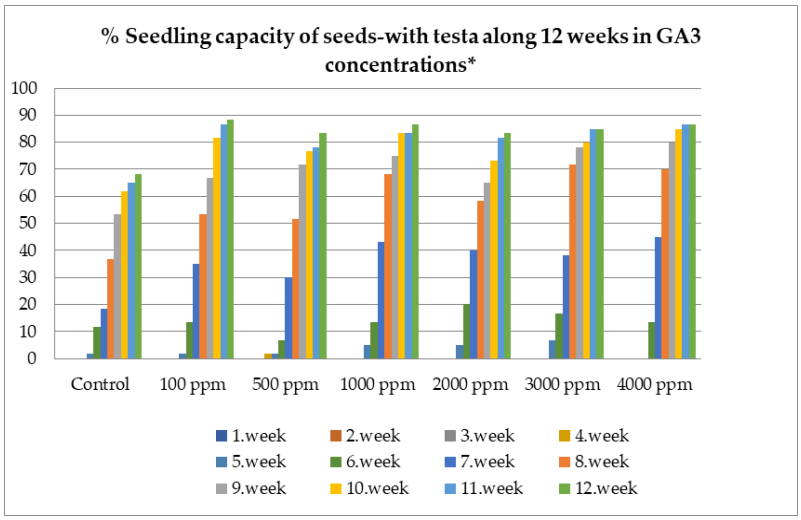

Figure 3. *Seedling capacity (%) of seeds-with testa along 12 weeks in GA_3 concentrations. (*Note that there is no seedling capacity in first 3 weeks)*

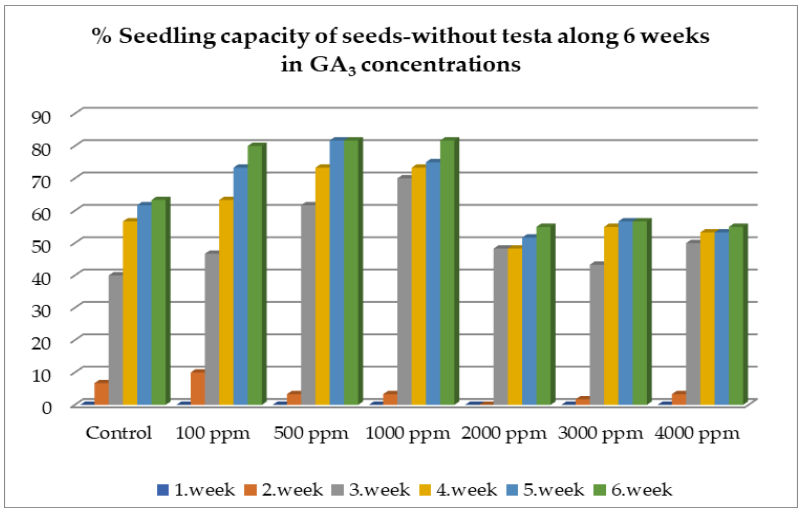

Figure 4. *Seedling capacity (%) of seeds-without testa along 6 weeks in GA_3 concentrations*

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Because of taking very different times in seedling growth of the two used seed types, the parameters on root number, maximum root length, average root length and shoot length were not compared with each other. In addition, when each is evaluated separately in terms of GA_3, only root number in seeds with testa (*Table 4*) and only maximum root length in seeds without testa (*Table 5*) showed statistical difference in the results.

Table 4. *Growth parameters of seedling from seeds with testa treated with different concentrations of GA_3 of Laurus nobilis L. after 12 weeks.*

GA_3 concentration	**Root number** (number/seedling)**	**Maximum root length* (cm/root/seedling)**	**Average root length* (cm/root/seedling)**	**Shoot length* (cm/shoot/seedling)**
Control	7.2 b	11.6	3.2	12.6
100 ppm	7.3 b	9.6	2.6	12.2
500 ppm	7.5 ab	11.5	2.9	12.5
1000 ppm	7.9 ab	9.9	2.6	13.2
2000 ppm	8.2 ab	11.5	2.8	11.9
3000 ppm	8.7 a	12.2	3.1	13.1
4000 ppm	8.2 ab	10.6	2.8	12.5
Average	**7.86**	**8.81**	**2.86**	**12.57**

*N.S., No significant difference in GA_3 concentrations in the parameters of seeds with testa
**Lower-case letters denote significant differences at the $p<0.05$ level in GA_3 concentrations in the parameter of seeds with testa

Table 5. *Growth parameters of seedlings from seeds without testa treated with different concentrations of GA_3 of Laurus nobilis L. after 6 weeks*

GA_3 concentration	**Root number* (number/seedling)**	**Maximum root length** (cm/root/seedling)**	**Average root length* (cm/root/seedling)**	**Shoot length* (cm/shoot/seedling)**
Control	4.5	8.8 ab	2.8	12.2
100 ppm	3.4	7.7 b	3.1	10.3
500 ppm	3.9	6.8 b	2.4	11.9
1000 ppm	4.1	10.5 a	3.9	13.1
2000 ppm	3.5	8.9 ab	3.9	11.3
3000 ppm	2.8	7.0 b	2.9	11.1
4000 ppm	2.6	6.6 b	3.2	10.3
Average	**3.54**	**8.04**	**3.17**	**11.46**

*N.S., No significant difference in GA_3 concentrations in the parameters of seeds-without testa
**Lower-case letters denote significant differences at the $p<0.05$ level in GA3 concentrations in the parameter of seeds-without testa

In seeds with testa, maximum root number reached 8.7 roots per seedling in 3000 ppm GA_3 treatment when control was 7.2 roots/seedling and 100 ppm GA_3 doses (7.3 roots/seedling) show minimum results. When maximum root length was observed in 1000 ppm GA_3 (10.5 cm/seedling) in seeds without testa, higher and lower doses than this showed lesser results. At the end of the seedling growth of seeds with testa, all GA_3 treatments statistically found useful on seedling capacity (88.3% in 100 ppm, 83.3% in

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500 ppm, 86.7% in 1000 ppm, 83.3% in 2000 ppm, 85.0% in 3000 ppm and 86.7% in 4000 ppm GA_3). But interestingly when seeds without testa were used; lesser doses of GA_3 found more useful for seedling capacity (80% in 100 ppm, 81.7% in 500 ppm and 1000 ppm GA_3) than higher doses (55.0% in 2000 ppm, 56.7% in 3000 ppm and 55.0% in 4000 ppm) and than control (63.3%) (*Table 6; Fig. 5*). Moreover, viability capacity of seedling from naked seed after 2 months of acclimatization tended to decrease gradually and statistically from control (95.6%) to 4000 ppm GA_3 (44.9%) when this parameter showed no difference in seeds with testa (*Table 6; Fig. 6*). In a previous study (Sari et al., 2006), when GA_3 was used in high dose (3000 ppm) seeds with testa showed lesser germination than 1000 ppm or control. Besides all, GA_3 and chemical scarification interaction success for *Cyclocarya palirus* (Fang et al., 2006) and for *Loelreuteria paniculata* (Rehman and Park, 2000) has also been demonstrated.

Table 6. *Seedling capacity at the end of the germinations and seedling viability capacity at the end of two months after acclimatization in seeds-with or without testa treated with different concentrations of GA_3 of Laurus nobilis L.*

GA_3 Concentration	**Seedling capacity*** (%)**		**Viability capacity*** (%)**	
	Seed with testa**	**Seed without testa****	**Seed with testa***	**Seed without testa****
Control	68.3 b A	63.3 ab A	92.3 A	95.6 a A
100 ppm	88.3 a A	80.0 a A	88.8 A	88.1 ab A
500 ppm	83.3 a A	81.7 a A	98.0 A	87.5 ab A
1000 ppm	86.7 a A	81.7 a A	96.2 A	87.9 ab A
2000 ppm	83.3 a A	55.0 b B	94.7 A	83.6 ab A
3000 ppm	85.0 a A	56.7 b B	98.3 A	60.1 ab B
4000 ppm	86.7 a A	55.0 b B	92.4 A	44.9 b B
Average	**83.09**	**67.63**	**94.39**	**78.24**

*N.S., No significant difference in GA3 concentrations in viability capacity in seeds with testa
**Lower-case letters denote significant differences at the $p<0.05$ level in GA3 concentrations in seedling capacity in seeds with testa, seeds without testa and viability capacity in seeds without testa
*** Capital letters denote significant differences at the $p<0.05$ level in seedling and viability capacity between seeds with testa and seeds without testa

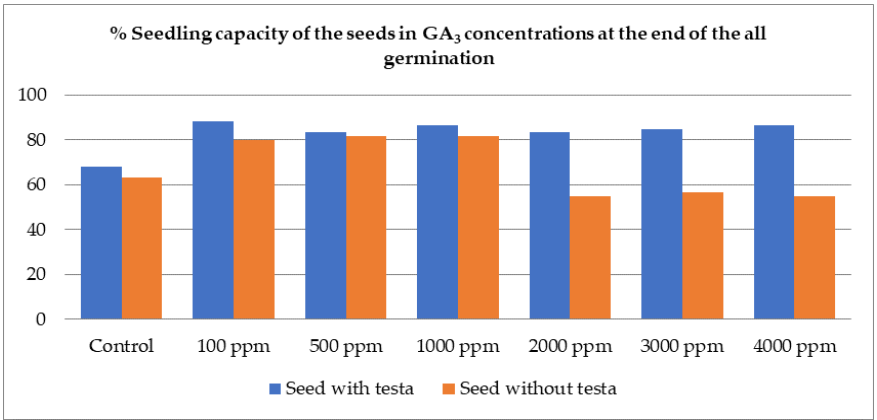

Figure 5. *Final seedling capacity (%) of seeds- with or -without testa in GA_3 concentrations*

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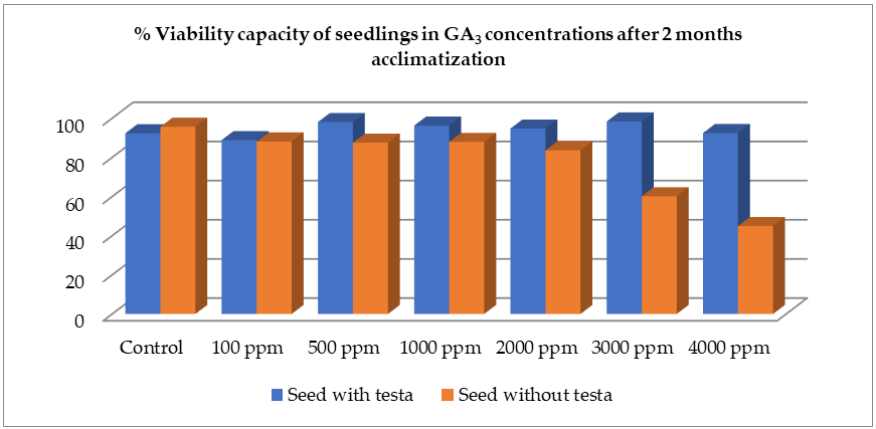

Figure 6. *Final viability capacity (%) of seeds-with or -without testa in GA_3 concentrations after 2 months acclimatization*

Conclusion

Although gibberellin is known in plant physiology and in agricultural practices as a crucial hormone or plant growth regulator from germination to death, the study did not showed effectiveness of this on average root and shoot length, even showed negative effects on germination and viability of seeds without testa under excessive concentrations. This may be due to plant species, gibberellin types, application methods, time and duration of treatments. When we evaluate the results of this study in general terms, it can be said that, if time in obtaining seedlings is the most important thing it may be better to use seeds without testa with relatively lesser GA_3, whereas if the aim is to get healthy seedlings a lot in number it may be better to use the seeds with testa in *Laurus nobilis* L.

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