

ARASTIRMA MAKALESİ / RESEARCH ARTICLE

REVERSAL OF ABSCISIC ACID INHIBITION IN SOME MONO- AND DICOT SEEDS BY STIMULATOR PLANT GROWTH REGULATORS

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ABSTRACT

Reversal of the inhibitory effect of abscisic acid (ABA) on seed germination and seedling growth by kinetin (KIN), benzyladenine (BA) and gibberellic acid (GA₃) was examined. Seeds of six wheat cultivars and two members of *Liliaceae* as monocotyl and six dicotyl species were germinated in Petri dishes containing ABA with BA and/or GA₃ at 20 °C in continuous darkness for 7 days. Cytokinins and cytokinin+GA₃ combinations were successful in overcoming the inhibition on germination in dicots. Particularly, BA alone and BA+GA₃ combinations were more successful than KIN. GA₃ alone was ineffective in dicots. In monocots, ABA inhibition of germination was reversed by cytokinin+GA₃ combinations. GA₃ was more successful than others for overcoming of ABA inhibition on the root elongation in monocot and dicot seeds. ABA inhibition on the hypocotyl elongation in dicot seeds reversed by cytokinin +GA₃ combinations and BA alone, whereas, in monocot seeds, only cytokinin + GA₃ combinations were successful in overcoming the ABA inhibition on the coleoptile elongation.

Keywords: Germination, Abscisic acid, Gibberellic Acid, Benzyladenine, Kinetin, Monocots, Dicots.

BAZI MONOKOTİL VE DİKOTİL TOHURLARINDA ABSISİK ASİT İNHİBİSYONUNUN STİMÜLATÖR BITKİ BÜYÜME MADDELERİ İLE YENİLMESİ

ÖZ

Absisik asit (ABA)'ın tohum çimlenmesi ve fide gelişimi üzerindeki engelleyici etkisinin Kinetin (KIN), Benziladenin (BA) ve Gibberellik asit (GA₃) tarafından yenilmesi araştırıldı. 2 *Liliaceae* üyesi ve 6 buğday çeşidi olmak üzere 8 monokotil ve 6 dikotil türün tohumları kullanıldı. Tohumlar, Petri kaplarında ABA ve BA, KIN ve/veya GA₃ varlığında 20 °C'de karanlıkta 7 gün çimlendirildi. Dikotillerde çimlenme üzerindeki ABA engellemesini yenmede sitokininler ve sitokinin + GA₃ kombinasyonları başarılıydı. Genellikle, dikotillerde BA ve BA + GA₃ kombinasyonları daha başarılıydı. Monokotillerde, çimlenme üzerindeki ABA engellemesi, sitokinin + GA₃ kombinasyonları tarafından yenildi. Monokotil ve dikotil tohumlarında ABA'nın kök uzamasını engelleyici etkisini yenmede GA₃ daha başarılıydı. Dikotil tohumlarında ise ABA'nın hipokotil uzamasını engellemesinin yenilmesinde sitokinin + GA₃ kombinasyonları başarılıydı.

Anahtar Kelimeler: Çimlenme, Absisik asit, Benziladenin, Kinetin, Gibberellik asit, Monokotil, dik.

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1. INTRODUCTION

Abscisic acid (ABA) plays regulatory roles in a host of physiological processes throughout plant growth and development. Seed germination, early seedling development, stomatal guard cell functions, and acclimation to adverse environmental conditions are key processes regulated by ABA (Leung and Giraudat, 1998). ABA is an inhibitor hormone existing in mature seeds, and plays an important role on the control of germination (Bewley and Black, 1983; Amen, 1968). ABA is a gibberellin antagonist and inhibits α -amylase synthesis which is stimulated by gibberellic acid in barley endosperm (Kramer and Kozłowski, 1979). Germination is inhibited by exogenous ABA in most nondormant seeds (Simpson and Naylor, 1962; Khan, 1971; Mayer and Poljakoff-Mayber, 1989; Lopez-Molina et al., 2001). The activity of ABA in seeds, is the regulation of gene expression particularly the induction of several different kinds of polypeptides and the inhibition of genes for certain reserve mobilizing enzymes and inhibition of protein and RNA synthesis for the elongation of radicle (Bewley and Black, 1982; Skriver and Mundy, 1990; Jacobsen and Chandler, 1987). ABA synthesis is required for the maintenance of dormancy. Control of dormancy in *Arabidopsis* is thought to result from the antagonistic effect of ABA and GAs on germination (Debeaujon and Koorneef, 2000).

Many dormant seeds can be stimulated to germinate with applied GA₃ (Amen 1968; Dhir and Sharma, 1991; Bradbeer, 1968; Jansen, 1980). Skoog and Armstrong (1970), noticed that in barley endosperm, the inhibition effect of ABA on α -amylase synthesis is affected by GA₃ more than KIN. The envelope in muskmelon and Cucurbitaceous seeds serves a semipermeable layer and act as primary barrier to radicle emergence (Wellbourn et al., 1995). According to a mechanism proposed for activities of kinetin (KIN), endosperm and integument limitings are decreased, while it stimulates proteinase and cellulase activities (Ikuma and Thimann, 1963a). In lettuce seed, KIN stimulates cell division which result in rupture of testa, while GA₃ stimulates the primary development signal, KIN overcomes the ABA inhibition, GA₃ overcomes the inhibition on shoot growth, while IAA is not effective (Houssa et al., 1990; Roy et al., 1973). In rice seeds, inhibition of exogenously applied ABA on germination and α -amylase synthesis, was reversed by both GA₃ and KIN individually, as well as in combinations (Rao et al., 1975).

The inhibition effects of ABA on germination has been studied for a long time (Addicott

and Lyon, 1968; Khan, 1971; Debaene-Gill et al., 1994; Chrispeels and Varner, 1966; Fountain and Bewley, 1976). There is a general inclination in the literature that ABA inhibition could be overcome by cytokinins, gibberellins were not successful (Khan, 1971; Murthy et al., 1988; Kabar, 1989; Khan and Downing, 1968; Debaene-Gill et al., 1994).

Some other investigations showed that, under the exogenous ABA inhibition, salt and temperature stress conditions, gibberellins are more effective in monocots, cytokinins are more effective in dicots (Garello and Page-Degivry, 1995; Sankhla and Sankhla, 1968; Rao et al., 1975; Hathway, 1990).

In order to test this opinions, the inhibition of germination and growth by exogenously applied ABA, in monocotyl and dicotyl seeds, investigated whether stimulator plant hormones GA₃, KIN and BA can reverse.

2. MATERIALS AND METHODS

2.1. Plant Material

In this work, eight monocots (of which six genera belonging to *Graminae* and two genera belonging to *Liliaceae*) and six dicot species were used. *Graminae* used in this work were *Triticum aestivum* L. var. *aestivum* (wheat), cultivars; Saraybosna, Atilla 12, MV20, Seri 82 and Flamura. *Liliaceae* seeds were *Allium cepa* L. (onion) cv. Early grand, *Allium porrum* Don. (leek) cv. İnegöl. Dicotyledonous taxa were *Medicago sativa* L. (clover) cv. Mesa sirsa, *Brassica oleracea* L. var. *oleracea* (cauliflower) cv. White cloud, *Brassica oleracea* L. var. *oleracea* (cabbage) cv. Yalova, *Lycopersicon esculentum* Miller (tomato) cv. Rio grande, *Raphanus sativus* L. (radish) cv. White radish, *Lactuca sativa* L. (lettuce) cv. Texas.

In these experiments, concentrations of ABA -which inhibit germination- were approximately 100, 75 and 50 per cent, as determined by pretreatments for each plant. The stimulatory hormone concentrations for reversal of ABA inhibition, were also determined by pretreatments. Those were: 300 μ M for GA₃, 50 μ M for BA and KIN.

2.2. Seed Germination

Seed germination was carried out in accordance with the methods proposed by Kabar (1990). Seeds were incubated for 7 days under 20°C continuous darkness. Seeds were surface

sterilized with 1% NaOCl for 15 min. And soaked in Petri dishes containing two layers of sterilized filter paper. 25 seeds were placed in each Petri dish with test solutions; all treatments were repeated three times. ABA, BA, IAA were purchased from Merck, GA₃ and KIN were purchased from Sigma. All the chemicals were dissolved in an appropriate solvent. Wheat seeds were soaked with 8 ml. The others were soaked with 6 ml of ABA and combinations of ABA and BA, KIN and GA₃ and also ABA+BA+ GA₃ and ABA+KIN+ GA₃ combinations. Germination was indicated as achieved when radicle length was to be at least 0.5 cm for *Graminae*, and 1mm radicle length of the *Liliaceae* and dicots. The percentage of germination and radicle/coleoptile/hypocotyl lengths were recorded everyday. LSD test was used to determine the statistical significance of differences among groups (Turan, 1995).

3. RESULTS AND DISCUSSION

Abscisic acid (ABA) plays regulatory roles in a host of physiological processes throughout plant growth and development. Seed germination, early seedling development, stomatal guard cell functions, and acclimation to adverse environmental conditions are key processes regulated by ABA. (Leung and Giraudat, 1998). Endogenous ABA levels change drastically during seed development, germination, and postgermination growth in response to developmental and environmental cues (Okamoto et al., 2006)

Germination percentage results showed the inhibition effects of ABA were reversed by cytokinins alone and cytokinin + GA₃ combinations (Table 1, 2). In dicot seeds, BA alone and combinations of BA + GA₃ treatments were successful. GA₃ alone did not show obvious effects (Table 1). GA₃ was the most successful treatment for overcoming the ABA inhibition on germination in monocots. Subsequent successful treatments were: BA + GA₃ and KIN + GA₃ combinations. BA was more successful than KIN (Table 2).

The inhibition effects of ABA on the root length of dicot seeds were reversed by GA₃ and KIN + GA₃ combinations (Fig. 1). The most successful treatment for dicot shoot growth was ABA+BA+ GA₃ combinations, BA and GA₃ alone were also successful (Fig. 2). ABA inhibition on the coleoptile length in wheat cultural varieties, was reversed by ABA+BA+ GA₃ combinations (Fig. 2). BA alone was more successful than GA₃ alone for most situations on coleoptile growth. In leek and onion, KIN +

GA₃ and BA + GA₃ combinations were generally more successful (Fig. 2).

During the germination of *Ginkgo* seeds, some plant endogenous hormones such as ABA, GA and Zeatin acted an important role in the every stage of seed dormancy and germination. GA₁₊₃ had a decisive role during dormancy and germination of *Ginkgo* seeds (Hua and Ju, 2006).

ABA might inhibit protein and nucleic acid synthesis (Ross and Bradbeer, 1971; Chen and Osborne, 1970; Fosket et al., 1981; Walton, 1980) and RNA synthesis (Chrispeels and Varner, 1976). The reversal of the ABA inhibition by cytokinins (Amen 1968; Ananiev et al., 1987) or gibberellin (Akazawa et al., 1988; Evins, 1971) could be associated with the increased RNA synthesis. Both cytokinins (Flores and Tobin, 1987; Fincher, 1989; Mosjidis and Zhang, 1995) and gibberellins (Fosket et al., 1981; Evenary and Mayer, 1954; Jarvis et al., 1968, Chrispeels and Varner, 1976) may reverse the ABA inhibition by increased polyribosome levels and protein synthesis. RNA and protein synthesis are essential for radicle elongation and these events inhibited by ABA.

Abscisic acid plays a variety of roles in plant growth and environmental response including a critical role in the development of seeds. A rise in ABA level during embryogenesis triggers processes that lead to the acquisition of desiccation tolerance and seed dormancy (Mohsin et al., 2006). The function of ABA may inhibit principle metabolic events following inhibition. ABA is believed to antagonize a positive GA signal that induces precocious germination and also suppresses maturation phase gene expression (White et al., 2000).

In typical Angiosperm seeds, such as tomato and *Arabidopsis*, germination requires both activation of embryo growth potential and weakening of a mechanical constraint such as testa or endosperm, both of which appear to be regulated by antagonistic actions of ABA and GA (Okamoto et al., 2006). The germination process and post-germination events are mediated by hormones, particularly GA and ABA. GA activation and ABA repression of hydrolase genes in the cereal aleurone are key processes that regulate the proper supply of nutrients from the endosperm to the embryo until the photosynthetic capability is completely acquired (Miguel et al., 2007).

Reactions of plants to different cytokinins may be different. Thomas et al.,

Table 1. Final germination percentages (mean \pm Standard deviation) of treated dicotyl seeds. (The mean values in each row followed by the same letter are not significantly different $LSD=5\%$)

| Dicot Cultivars | ABA Concentrations | Phytohormone Treatments | | | | | |
|-----------------------------|--------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|---|--|
| | | ABA (Control) | 50 μ M BA | 50 μ M KIN | 300 μ M GA ₃ | 50 μ M BA + 300 μ M GA ₃ | 50 μ M KIN + 300 μ M GA ₃ |
| Cabbage cv. Yalova | 300 μ M ABA | 6.7 ^b \pm 1.9 | 36.0 ^a \pm 4.9 | 37.3 ^a \pm 3.8 | 29.3 ^{ab} \pm 3.3 | 32.0 ^a \pm 5.6 | 36.0 ^a \pm 6.5 |
| | 150 μ M ABA | 36.0 ^a \pm 3.2 | 38.7 ^a \pm 6.5 | 45.3 ^a \pm 4.9 | 38.7 ^a \pm 3.2 | 50.7 ^a \pm 6.8 | 37.3 ^a \pm 3.3 |
| | 80 μ M ABA | 57.3 ^a \pm 4.9 | 53.3 ^a \pm 7.5 | 42.7 ^a \pm 3.8 | 46.7 ^a \pm 8.2 | 49.3 ^a \pm 1.9 | 46.7 ^a \pm 6.8 |
| Cauliflower cv. White cloud | 150 μ M ABA | 1.3 ^b \pm 0.9 | 24.0 ^a \pm 3.3 | 26.7 ^a \pm 1.9 | 32.0 ^a \pm 3.3 | 26.7 ^a \pm 1.9 | 24.0 ^a \pm 3.3 |
| | 70 μ M ABA | 25.3 ^b \pm 1.9 | 34.7 ^{ab} \pm 9.4 | 25.4 ^b \pm 1.9 | 48.0 ^a \pm 1.9 | 53.3 ^a \pm 1.9 | 44.0 ^{ab} \pm 6.8 |
| | 50 μ M ABA | 61.3 ^{ab} \pm 1.9 | 58.7 ^{ab} \pm 9.9 | 44.0 ^b \pm 5.7 | 65.3 ^a \pm 4.9 | 54.6 ^{ab} \pm 6.8 | 45.3 ^b \pm 4.9 |
| Clower cv. Mesa sirsa | 150 μ M ABA | 2.7 ^d \pm 1.9 | 50.7 ^a \pm 1.9 | 28.0 ^{abc} \pm 3.8 | 24.0 ^{bcd} \pm 4.9 | 45.3 ^a \pm 5.6 | 26.7 ^{cd} \pm 6.8 |
| | 100 μ M ABA | 24.0 ^c \pm 3.3 | 34.7 ^{ab} \pm 9.4 | 25.4 ^b \pm 1.9 | 48.0 ^a \pm 1.9 | 53.3 ^a \pm 1.9 | 44.0 ^{ab} \pm 6.8 |
| | 30 μ M ABA | 24.0 ^c \pm 3.3 | 48.0 ^b \pm 3.8 | 28.0 ^c \pm 3.3 | 29.3 ^c \pm 1.9 | 65.3 ^a \pm 1.9 | 28.0 ^c \pm 3.3 |
| Tomatoes cv. Rio grande | 35 μ M ABA | 0.0 ^e \pm 0.0 | 45.3 ^{ab} \pm 8.6 | 2.7 ^e \pm 3.8 | 57.3 ^a \pm 4.9 | 57.3 ^a \pm 17.9 | 4.0 ^{bc} \pm 5.7 |
| | 25 μ M ABA | 20.0 ^c \pm 0.0 | 62.6 ^{ab} \pm 0.9 | 21.3 ^c \pm 4.9 | 64.7 ^a \pm 3.2 | 82.7 ^a \pm 1.9 | 34.7 ^{bc} \pm 1.9 |
| | 10 μ M ABA | 37.3 ^b \pm 3.8 | 79.3 ^a \pm 8.4 | 40.0 ^b \pm 0.0 | 82.7 ^a \pm 1.9 | 86.0 ^a \pm 3.8 | 58.7 ^{ab} \pm 7.5 |
| Radish cv. White radish | 600 μ M ABA | 2.7 ^e \pm 3.8 | 41.3 ^{ab} \pm 4.9 | 21.3 ^{bc} \pm 6.8 | 17.3 ^c \pm 1.9 | 50.7 ^a \pm 4.9 | 44.0 ^b \pm 6.8 |
| | 400 μ M ABA | 22.7 ^d \pm 1.9 | 56.0 ^a \pm 8.6 | 40.0 ^{bc} \pm 0.0 | 37.3 ^{cd} \pm 7.5 | 60.0 ^a \pm 6.8 | 53.3 ^{ab} \pm 4.9 |
| | 300 μ M ABA | 44.0 ^d \pm 0.0 | 65.3 ^b \pm 4.9 | 52.0 ^{cd} \pm 6.5 | 54.7 ^c \pm 3.8 | 86.7 ^a \pm 7.5 | 74.7 ^b \pm 1.9 |
| Lettuce cv. Texas | 250 μ M ABA | 0.0 ^e \pm 0.0 | 40.0 ^b \pm 3.3 | 20.0 ^{bc} \pm 3.3 | 25.3 ^{bc} \pm 5.8 | 69.3 ^a \pm 4.9 | 24.0 ^{bc} \pm 5.7 |
| | 150 μ M ABA | 18.7 ^c \pm 1.9 | 82.7 ^a \pm 6.8 | 24.0 ^c \pm 5.7 | 21.3 ^c \pm 1.9 | 92.0 ^a \pm 8.6 | 60.0 ^b \pm 5.7 |
| | 50 μ M ABA | 45.3 ^c \pm 3.8 | 89.3 ^b \pm 6.8 | 88.0 ^b \pm 5.7 | 40.0 ^c \pm 0.0 | 94.7 ^{ab} \pm 1.9 | 100.0 ^a \pm 0.0 |

Table 2. Final germination percentages (mean \pm Standard deviation) of treated monocotyl seeds. (The mean values in each row followed by the same letter are not significantly different LSD= 5 %)

| Monocot Cultivars | ABA Concentrations | Phytohormone Treatments | | | | | | |
|-----------------------|--------------------|------------------------------|------------------------------|------------------------------|-------------------------------|---|---|--|
| | | ABA (Control) | 50 μ M BA | 50 μ M KIN | 300 μ M GA ₃ | 50 μ M BA + 300 μ M GA ₃ | 50 μ M KIN +300 μ M GA ₃ | |
| Wheat cv. Saraybosna | 300 μ M ABA | 6.7 ^b \pm 4.9 | 20.0 ^{ab} \pm 1.8 | 6.7 ^b \pm 4.9 | 39.3 ^a \pm 4.9 | 28.0 ^{ab} \pm 1.8 | 18.6 ^{ab} \pm 1.9 | |
| | 200 μ M ABA | 20.0 ^{bc} \pm 0.0 | 33.3 ^{ab} \pm 4.9 | 18.7 ^c \pm 4.9 | 29.3 ^{abc} \pm 4.9 | 36.0 ^a \pm 1.8 | 30.7 ^{abc} \pm 8.2 | |
| | 100 μ M ABA | 36.0 ^a \pm 0.0 | 42.7 ^a \pm 3.7 | 37.3 ^a \pm 3.8 | 52.0 ^a \pm 5.6 | 50.7 ^a \pm 15.1 | 45.3 ^a \pm 1.9 | |
| Wheat cv. Flamura | 300 μ M ABA | 0.0 ^c \pm 0.0 | 20.0 ^b \pm 3.3 | 1.3 ^c \pm 1.9 | 38.7 ^a \pm 4.9 | 20.0 ^b \pm 3.3 | 26.7 ^{ab} \pm 1.9 | |
| | 250 μ M ABA | 20.0 ^c \pm 3.3 | 28.0 ^{bc} \pm 3.3 | 25.3 ^{bc} \pm 4.9 | 30.6 ^{bc} \pm 5.7 | 57.3 ^a \pm 3.7 | 36.7 ^{ab} \pm 3.4 | |
| | 120 μ M ABA | 40.0 ^a \pm 0.0 | 44.0 ^a \pm 5.7 | 44.0 ^a \pm 5.7 | 53.3 ^a \pm 1.9 | 50.7 ^a \pm 1.8 | 41.3 ^a \pm 1.9 | |
| Wheat cv. MV 20 | 250 μ M ABA | 5.3 ^b \pm 4.9 | 26.3 ^a \pm 5.6 | 5.3 ^b \pm 4.9 | 32.0 ^a \pm 6.5 | 37.3 ^a \pm 4.9 | 30.6 ^a \pm 4.9 | |
| | 100 μ M ABA | 21.3 ^c \pm 1.8 | 52.0 ^{ab} \pm 8.6 | 26.3 ^{bc} \pm 6.7 | 62.6 ^a \pm 5.6 | 46.6 ^{abc} \pm 5.7 | 32.0 ^{bc} \pm 5.6 | |
| | 60 μ M ABA | 45.3 ^{ab} \pm 4.9 | 46.6 ^{ab} \pm 3.8 | 40.0 ^b \pm 3.2 | 58.6 ^a \pm 7.5 | 41.3 ^b \pm 4.9 | 45.3 ^{ab} \pm 10.4 | |
| Wheat cv. Seri 82 | 250 μ M ABA | 6.6 ^d \pm 1.8 | 18.6 ^b \pm 8.2 | 8.0 ^{cd} \pm 3.2 | 32.0 ^a \pm 5.6 | 21.3 ^b \pm 4.9 | 17.3 ^{bc} \pm 1.8 | |
| | 100 μ M ABA | 24.0 ^a \pm 3.2 | 49.3 ^a \pm 6.5 | 36.0 ^a \pm 3.7 | 56.0 ^a \pm 3.6 | 53.3 ^a \pm 5.7 | 45.3 ^a \pm 5.7 | |
| | 60 μ M ABA | 44.0 ^a \pm 5.6 | 49.3 ^a \pm 6.5 | 40.0 ^a \pm 3.8 | 52.0 ^a \pm 5.6 | 50.6 ^a \pm 9.4 | 54.6 ^a \pm 1.9 | |
| Wheat cv. Gönen | 300 μ M ABA | 5.3 ^b \pm 3.7 | 33.3 ^a \pm 9.4 | 18.6 ^{ab} \pm 3.2 | 33.3 ^a \pm 4.9 | 29.3 ^a \pm 1.8 | 17.3 ^{ab} \pm 1.8 | |
| | 250 μ M ABA | 22.6 ^b \pm 1.8 | 22.6 ^b \pm 1.8 | 20.0 ^b \pm 3.2 | 50.6 ^a \pm 4.9 | 45.3 ^{ab} \pm 4.9 | 34.6 ^{ab} \pm 6.7 | |
| | 150 μ M ABA | 38.6 ^a \pm 3.7 | 54.6 ^a \pm 9.4 | 38.6 ^a \pm 3.7 | 56.0 ^a \pm 5.6 | 65.3 ^a \pm 5.7 | 42.0 ^a \pm 4.9 | |
| Wheat cv. Atilla 12 | 250 μ M ABA | 5.3 ^d \pm 4.9 | 22.6 ^{bc} \pm 7.5 | 12.0 ^{cd} \pm 5.6 | 45.3 ^a \pm 7.5 | 24.0 ^{bc} \pm 8.6 | 28.0 ^{ab} \pm 6.5 | |
| | 100 μ M ABA | 25.3 ^c \pm 3.7 | 40.0 ^{bc} \pm 3.2 | 28.6 ^c \pm 2.4 | 69.3 ^a \pm 9.9 | 54.6 ^{ab} \pm 5.6 | 36.0 ^c \pm 3.2 | |
| | 50 μ M ABA | 41.3 ^c \pm 7.5 | 45.3 ^c \pm 5.7 | 42.6 ^c \pm 4.9 | 64.0 ^{ab} \pm 5.6 | 70.0 ^a \pm 3.9 | 46.6 ^{bc} \pm 4.9 | |
| Leek cv. İnegöl | 65 μ M ABA | 1.3 ^e \pm 1.9 | 10.7 ^{ab} \pm 1.9 | 4.0 ^{bc} \pm 3.3 | 6.7 ^{abc} \pm 3.8 | 14.7 ^a \pm 6.8 | 5.3 ^{bc} \pm 1.9 | |
| | 45 μ M ABA | 20.0 ^b \pm 0.0 | 20.0 ^b \pm 0.0 | 20.0 ^b \pm 0.0 | 22.7 ^a \pm 1.9 | 21.3 ^{ab} \pm 1.9 | 20.0 ^b \pm 0.0 | |
| | 10 μ M ABA | 38.7 ^a \pm 1.9 | 44.0 ^a \pm 3.3 | 36.0 ^a \pm 3.3 | 40.0 ^a \pm 5.6 | 38.7 ^a \pm 1.9 | 33.3 ^a \pm 1.9 | |
| Onion cv. Early grand | 1000 μ M ABA | 8.0 ^e \pm 0.0 | 22.7 ^{ab} \pm 6.8 | 14.7 ^{bc} \pm 3.8 | 33.3 ^a \pm 4.6 | 22.8 ^{ab} \pm 3.8 | 25.3 ^{ab} \pm 7.5 | |
| | 500 μ M ABA | 30.7 ^{bc} \pm 1.9 | 50.7 ^a \pm 3.8 | 30.7 ^c \pm 6.8 | 45.3 ^{ab} \pm 7.5 | 53.3 ^a \pm 9.9 | 46.0 ^a \pm 9.4 | |
| | 350 μ M ABA | 42.7 ^c \pm 4.9 | 60.0 ^a \pm 5.7 | 48.0 ^{bc} \pm 3.2 | 50.7 ^{bc} \pm 1.9 | 54.7 ^{ab} \pm 6.8 | 45.3 ^{bc} \pm 4.9 | |

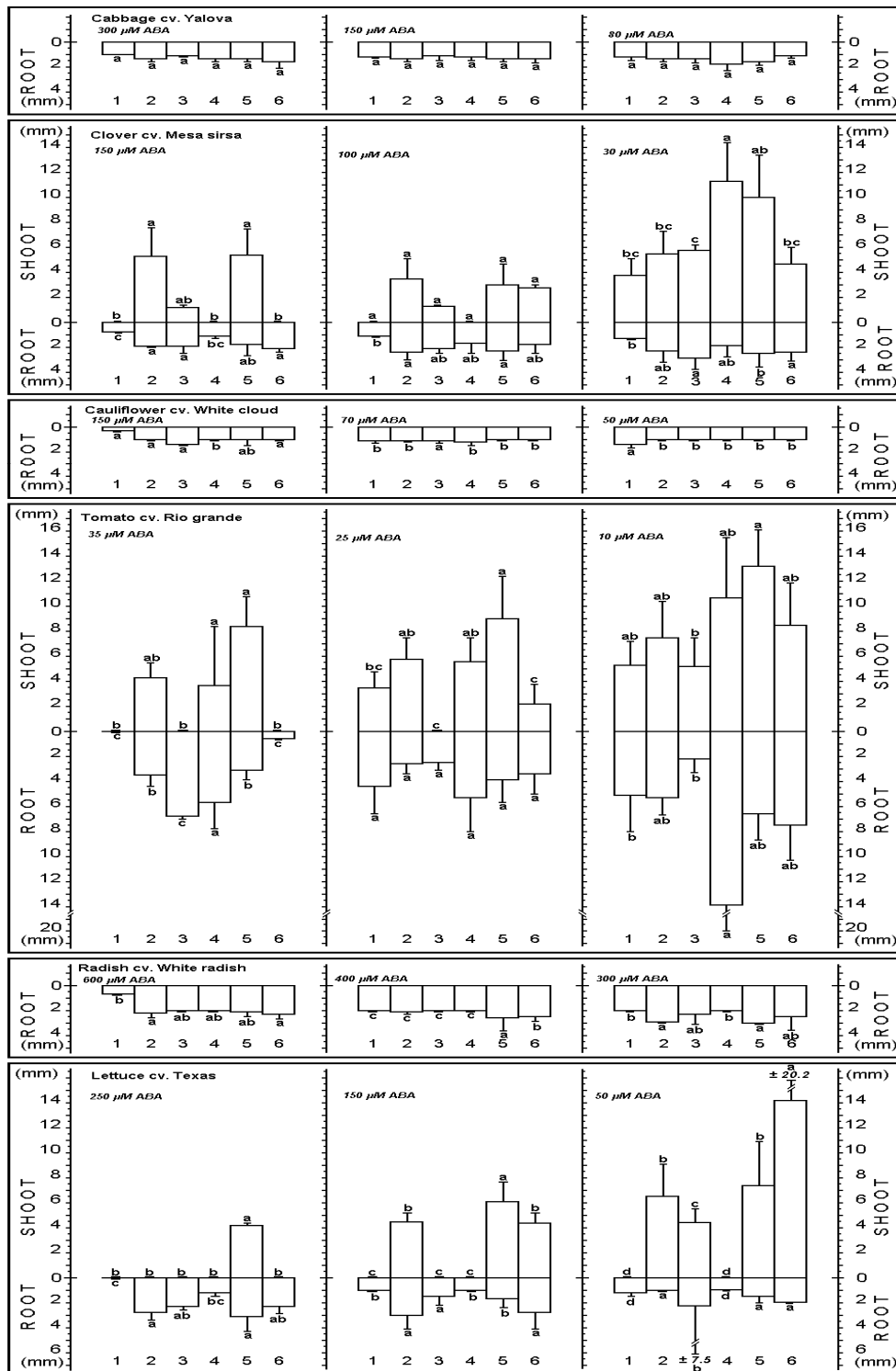


Figure 1. The average root and shoot lengths of dicot seeds (mm). Three different ABA concentrations for each plant are shown on the figure. Numbers, show different treatments for reversal of the ABA inhibition, 1: ABA (control), 2: ABA + 50 μM BA, 3: ABA + 50 μM KIN, 4: ABA + 300 μM GA₃, 5: ABA + 50 μM BA + 300 μM GA₃, 6: ABA + 50 μM KIN + 300 μM GA₃ (The values indicated as columns within each row followed by the same letter are not significantly different, LSD= 5 %, vertical bars on the columns represent standard deviation of the means)

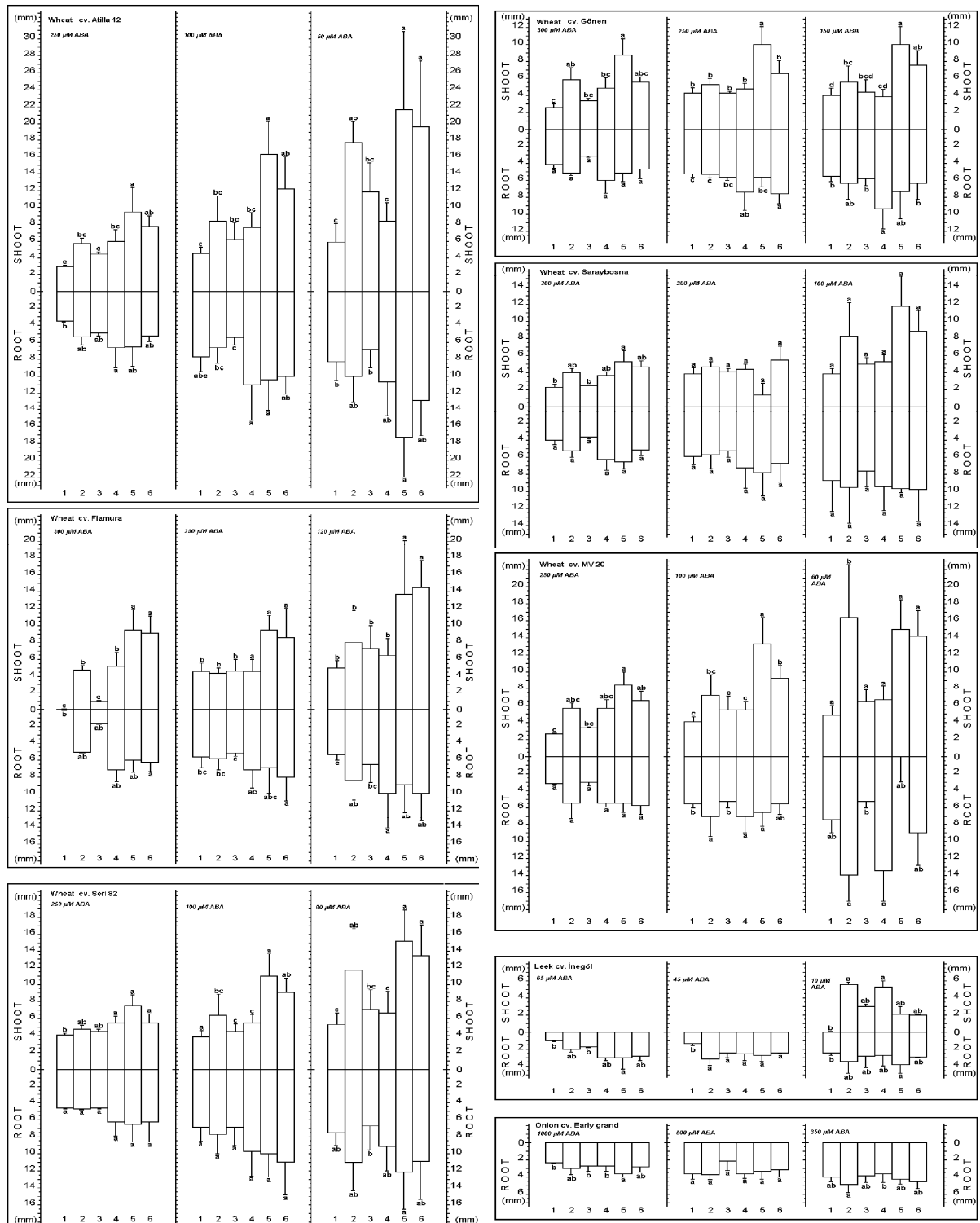


Figure 2. The average root and shoot lengths of monocotyl seeds (mm). Three different ABA concentrations for each plant are shown on the figure. Numbers, show different treatments for reversal of the ABA inhibition, 1: ABA (control), 2: ABA + 50 µM BA, 3: ABA + 50 µM KIN, 4: ABA + 300 µM GA3, 5: ABA + 50 µM BA + 3 00 µM GA3, 6: ABA + 50 µM KIN + 300 µM GA3 (The values indicated as columns within each row followed by the same letter are not significantly different, LSD= 5 %, vertical bars on the columns represent standart deviation of the means)

(1975), showed that, BA is more effective than other cytokinins on the breaking of dormancy in celery seeds. We also found that BA was more effective than KIN in tomato, lettuce and clover seeds, as well as most of the monocot seeds.

Amen (1968), suggested gibberellin activities are related with degradation of food reserves and cytokinin activities are related with beginning of cell proliferation. The synergistic relationship between cytokinins and gibberellins have also been discussed and observed in data from our experiments. In dicot seeds, GA₃ was generally less effective than BA + GA₃ combinations on the ABA inhibition (Table 1).

Kabar (1990), reported that GA₃ was far more effective than cytokinins and KIN was not effective in some *Graminae* and *Liliaceae* seeds. Dormant or nondormant seeds belonging the same or different species may involve different levels of gibberellin, cytokinin or inhibitors (Kabar, 1989;1990; Skoog and Armstrong, 1970). Stimulation of germination by GA₃ is occurred via synthesis of α -amylase and other hydrolases. ABA inhibits all this enzyme synthesis (Chrispeels and Varner, 1976; Khan, 1971; Ikuma and Thimann, 1963b; Debaene-Gill et al., 1994; Thomas et al., 1975). Synergistic relationship between stimulator hormones have previously been reported before (Khan and Downing, 1968; Kabar and Baltepe, 1990). Khan and Downing (1968), reported that KIN + GA₃ combinations were more successful than they were alone. In several experiments of the present work, BA + GA₃ combinations were more effective than KIN + GA₃ combinations. Sankhla and Sankhla (1968), reported that ABA inhibition was reversed by KIN more than GA₃, but in the condition of GA₃ existence, ABA could not show its inhibition effect.

Present study show that KIN was not successful on the overcoming the ABA inhibition, while BA was successful in most conditions in dicots (Table 1, Figure 1). It was previously reported that, in *Lemna minor*, BA reversed the ABA inhibition (Murthy et al., 1988). Unfortunately the studies leading to this opinion, are scant. According to Ikuma and Thimann (1963a), although KIN increased germination it was not effective when existed alone, but GA₃ can stimulate germination alone.

Therefore, it seems that plant hormones may play permissive roles during germina-

tion and can regulate the processes involved in germination and growth. Most of the recent literature are concerned with the effects of ABA on gene regulation (Okamoto et al., 2006; Lopez- Molina et al., 2001; Cadman et al., 2006). ABA, as an inhibitor plant hormone, inhibited germination in most situations and this inhibition could be overcome by cytokinin + GA₃ combinations in both mono and dicots. It has been found that, BA may be far more effective than KIN at the same concentration levels (50 μ M).

Results from this research and others could help us to evaluate and understand the role of hormones on seed dormancy.

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REFERENCES

- Addicott, F.T., and Lyon, J.L. (1868). Physiology of abscisic acid and related substances. *Ann. Rev. Plant Physiol.* 359–384.
- Akazawa, T., Mitsui, T. and Hawashi, M. (1988). Recent progress in alpha amylase biosynthesis, In: *The Biochemistry of Plants*. Preiss, J. (ed.), Academic Press. San Diego.
- Amen, R.D. (1968). A model of seed dormancy. *Bot. Rev.* 34,1–31.
- Ananiev, E.D., Karagyozov, L.K. and Karanov, E.N. (1987). Effect of cytokinins on ribosomal RNA expression in excised cotyledons of *Cucurbita pepo* L. *Planta* 170, 370–378.
- BangHua, C. and ChunJu, C. (2006). Study on the after-ripening physiology and endogenous hormones of Ginkgo biloba seeds. *Scientia Silvae Sinicae* 42, 32–37.
- Bewley, J.D. and Black, M. (1982). *Physiology and Biochemistry of Seeds, in Relation to Germination*. V 2. Springer, Verlag, Berlin. Heiderberg, New York 206–269.

- Bewley, J.D. and Black, M. (1983). Physiology and Biochemistry of Seeds, in Relation to Germination. V 1. Springer, Verlag, Berlin, Heiderberg, New York 81–100.
- Biddington, N.L. and Thomas, T.H. (1976). Influence of different cytokinins on the germination of lettuce (*Lactuca sativa*) and celery (*Apium graveolens*) seeds, *Physiol. Plant.* 37, 12–16.
- Bradbeer, J.W. (1968). Studies in the seed dormancy, the role of endogenous inhibitors and gibberellin in the dormancy and germination of *Corylus avellana* L. seeds. *Planta* 78, 266–276.
- Cadman, C.S.C., Toorop, P.E., Hilhors, t H. W.M. and Finch-Savage, W.E. (2006). Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J.* 46, 805–822.
- Chen, D. and Osborne, D.J. (1970). Hormones in the translational Ccontrol of early germination in wheat embryos. *Nature* 226, 1157–1160.
- Chrispeels, M.J. and Varner, J.E. (1976). Hormonal control of enzyme synthesis: on the mod of action of gibberellic acid and abscisin in aleurone layers of Barley. *Plant Physiol.* 42, 1008–1016.
- Chrispeels, M.J. and Varner, J.E. (1966). Inhibition of gibberellic acid induced formation of α amylase by abscisin II. *Nature* 212, 1066–1067.
- Debaene-Gill S., Allen P.S. and White D.B. (1994). Dehydration of germinating perennial ryegrass can alter rate of subsequent radicle emergence. *J. Exp. Bot.* 45, 1301–1307.
- Debeaujon, I., Koornneef, M. (2000). Gibberellin requirements for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiol.* 122, 415–424.
- Dhir, K.K. and Sharma, R. (1991). ABA caused inhibition of seedling growth and its removal by kinetin. *New Trends in Plant Physiol.* 71–73.
- Eady, F.C. and Eata, G.W. (1972). The role of gibberellic acid and gibberellin like substances in the dormancy of cranberry. *Canad. J. Plant Sci.* 52, 263–271.
- Evenary, M. and Mayer, A. (1954). The effect of auxin on germination of lettuce seeds. *Bull. Res. Council. Israel* 4, 81–84.
- Evins, W.H. (1971). Enhancement of polyribosome formation and induction of tryptophan-rich proteins by gibberellic acid. *Biochem.* 10, 4295–4303.
- Fincher, G.B. (1989). Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Ann. Rev. Plant Physiol.* 40, 305–346.
- Flores, S. and Tobin, E.M. (1987). Benzyladenine regulation of the expression of two nuclear genes for chloroplast proteins, In: Molecular Biology of Plant Growth Control, Fox, J.E., and Jacobs, M. (eds.). Alan R. Liss. New York.
- Fosket, D.E., Morejohn, L.C. and Wasterling, K.E. (1981). Control of growth by cytokinin: an examination of tubulin synthesis during cytokinin-induced growth in cultured cells of paul's scarlett rose. In: Metabolism and Molecular Activities of Cytokinins, Guern, J., and Peaud-Lenoel C. (eds.) Springer-Verlag, Berlin.
- Fountain, D.W. and Bewley J.D. (1976). Modulation of pregermination protein synthesis by gibberellic acid, abscisic acid and cytokinin. *Plant Physiol.* 58, 530–536.
- Garello, G. and Le Page-Degivry, M.T. (1995). Desiccation-sensitive *Hoepa odorata* seeds: sensitivity to abscisic acid, water potential and inhibitors of protein synthesis, *Physiol. Plant.* 95, 45–50.
- Hathway, D.E. (1990). Plant growth and development in molecular perspective. *Biol. Rev.* 65, 473–515.
- Houssa, C., Jacquard A. and Bernier G. (1990). Application of replicon origins as a possible target for cytokinins in shoot meristems of *Sinapsis*. *Planta* 181, 324–326.
- Ikuma, H. and Thimann, K.V. (1963a). The action of kinetin and photosensitive lettuce seed as compared with gibberellic acid. *Plant Cell Physiol.* 4, 113–128.
- Ikuma, H. and Thimann, K.V. (1963b). The role of the seed coats in germination of photo-

- sensitive lettuce seeds. *Plant Cell Physiol.* 4, 169–183.
- Jacobsen, J.V., Chandler, P.M. (1987). Gibberellin and abscisic acid in germinating cereals In: Davies PJ, ed. *Plant Hormones and their role in plant growth and development*. Boston, Martinus, Nijhoff. 164–193.
- Jansen, H. (1980). Bahçe Ziraatında büyüücü ve engelleyici maddelerin kullanılması ve Önemi. Trans. Gülerüz M. Atatürk Üniversitesi Ziraat Fakültesi Yayınları No: 279, Erzurum. 325–28 (In Turkish).
- Jarvis, B.C., Frankland, J.H., and Cherry, J.H. (1968). Increased nucleic acid synthesis in relation to the breaking of dormancy of hazel seed by gibberellic acid. *Planta* (Berl.) 83, 257–266.
- Kabar, K. (1990). Comparison of kinetin and gibberellic acid effects on seed germination under saline conditions, *Phyton* (Horn) 30, 291–298.
- Kabar, K. and Baltepe, Ş. (1990). Effects of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on germination of barley and lettuce seeds. *Phyton*(Horn) 30, 65–74.
- Kabar, K. (1989). Interactions among salt (NaCl), kinetin and gibberellic acid in the germination of lettuce seeds. *Tr. J. Bot.* 13, 296–300.
- Khan, A.A. (1971). Cytokinins, permissive roles in seed germination. *Science* 171, 853–859.
- Khan, A.A. and Downing, R.D. (1968). Cytokinin reversal of abscisic acid inhibition of growth and α -amylase synthesis in barley seed. *Physiol. Plant.* 21, 1301–1307.
- Kramer, P.J. and Kozlovski, T.T. (1979). *Physiology of Woody Plants*. Academic Press, New York.
- Leung, J., Giraudat, J. (1998). Abscisic acid signal transduction. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 49, 199–222.
- Lopez-Molina, L., Mongrand, S., Chua, N.H. (2001). A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. *PNAS* 98, 4782–4787.
- Mayer, A.M. and Poljakoff-Mayber, A. (1989). *The Germination of Seeds*. Pergamon Oxford.
- Mohsin, T., Khan, N., Naqvi, F.N. (2006). Effects of exogenous plant growth regulators on embryonic development of *Vigna radiata* (Mung bean): Differential expression of amylase in immature embryos cultured in vitro. *Pakistan J. Biological Sci.* 9, 160–163.
- Moreno-Risueno, M. A., Di'az, I., Carrillo, L., Fuentes, R. and Carbonero, P. (2007). The HvDOF19 transcription factor mediates the abscisic acid dependent repression of hydrolase genes in germinating barley aleurone. *Plant J.* 51, 352–365.
- Mosjidis, J.A. and Zhang, X. (1995). Seed germination and root growth of several Vicia species at different temperatures. *Seed Sci. Tech.* 23, 749–759.
- Murthy, B.N.S., Murti, G.S.R. and Reddy, Y.N. (1988). Effects of abscisic acid on seed germination and its endogenous levels in ber (*Zizyphus mauritiana*) seeds. *Plant Physiol.* 32, 275–277.
- Okamoto, M., Kuwahara, A., Seo M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshiba, T. and Nambara, E. (2006) CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. *Plant Physiol.* 141, 97–107.
- Rao, V.S., Sankhla, N. and Khan, A.A. (1975). Additive and synergistic effect of kinetin and ethrel on germination, thermodormancy and polyribosome formation in lettuce seeds. *Plant Physiol.* 56, 263–266.
- Ross, J.D., and Bradbeer, J.W. (1971). Studies in seed dormancy, the content of exogenous gibberellins in *Corylus avellana* L. *Planta*(Berl) 100, 288–302.
- Roy, T., Ghose, B. and Sircor, M. (1973). Cyclic AMP promotion and abscisic acid inhibition of α -amylase activity in the seeds of Rice (*Oryza sativa* L.). *J. Exp. Bot.* 24, 1064–1068.
- Sankhla, N. and Sankhla, D. (1968). Reversal of abscisic acid induced inhibition of lettuce

seed germination and seedling growth by kinetin. *Physiol. Plant.* 21,190–195.

Simpson, G.M. and Naylor, M. (1962). Dormancy studies In seeds of *Avena fatua*. A relationship between maltase amylases and gibberellin. *Canad. J. Bot.* 40,1659–1673.

Skoog, F. and Armstrong, D. (1970). Cytokinins. *Ann. Rev. Plant Physiol.* 359–384.

Skriver, K. and Mundy, J. (1990). Gene expression in response to abscisic acid and osmotic stress. *The Plant Cell* 2, 503–512.

Thomas, T.H., Palevitch, D., Biddington, N.L. and Austin, R.B. (1975), Growth regulators and the phytochrom-mediated dormancy of celery seeds. *Physiol. Plant.* 35,101–106.

Turan, Z.M. (1995). Araştırma ve Deneme Metodları. Uludağ Üniversitesi Ziraat Fakültesi Ders Notları, Uludağ Üniversitesi Basımevi, Bursa (In Turkish).

Walton, D.C. (1980). Biochemistry and physiology of abscisic acid . *Ann. Rev. Plant Physiol.* 31, 453–489.

Wellbourn, G.E., Muthui, W.J., Wilson, J.H., Grayson, J.L. and Fell, R.D. (1995). Weakening of muskmelon perisperm envelope tissue during germination . *J. Exp. Bot.* 46,391–400.

White, C.N., Proebsting, W.M., Hedden, P. and Rivin, C.J. (2000). Gibberellins and seed development in maize. I. Evidence that Gibberellins/ Abscisic acid balance governs germination versus maturation pathways. *Plant Physiol.* 122, 1081–1088.



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