

Effects of Gibberellic Acid Treatment for Pollen Sterility Induction on the Physiological Activity and Endogenous Hormone Levels of the Seed in Safflower

Hasan BAYDAR

Süleyman Demirel University, Faculty of Agriculture, Department of Field Crops, 32260. Isparta - TURKEY

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Abstract: In this research, our aim was to determine the effects of gibberellic acid, which was applied to safflower plants (*Carthamus tinctorius* L. cv. Diñer 5-118) for pollen sterility induction, on some physiological activity and endogenous hormone levels of the seeds. Exogenously applied gibberellic acid (GA₃) strongly influenced the endogenous hormone levels of the seeds by decreasing the levels of GA₃ and zeatin, and increasing the levels of indole-3-acetic acid (IAA) and abscisic acid (ABA). The lowered endogenous GA₃/ABA and zeatin/IAA ratios in the seeds significantly decreased the germination percentage and hypocotyl elongation, respectively. The seeds from GA₃ treated plants had more hull percentage and less oil content than seeds from the non-GA₃ treated plants. As a consequence, it was indicated that poor germination and emergence vigor might be a major problem in hybrid safflower seeds produced from plants treated with gibberellic acid.

Key Words: Safflower, *Carthamus tinctorius* L., Seed physiology, Gibberellic acid, Plant hormones, Pollen sterility

Aspirde Polen Kısırlık Uyarısı İçin Gibberellik Asit Uygulamasının Tohumun Fizyolojik Aktivitesi ve İçsel Hormon Seviyeleri Üzerine Etkisi

Özet: Bu araştırma, polen kısırlığı yaratmak için aspir bitkisine (*Carthamus tinctorius* L. cv. Diñer 5-118) uygulanan gibberellik asidin tohumun bazı fizyolojik aktiviteleri ve içsel hormon seviyeleri üzerindeki etkilerini belirlemek amacıyla gerçekleştirilmiştir. Dıştan bitkilere uygulanan gibberellik asit (GA₃) tohumların içsel GA₃ ve zeatin seviyelerinde azalışlara, indol-3-asetik asit (IAA) ve absisik asit (ABA) seviyelerinde artışlara neden olarak, içsel hormon seviyeleri üzerinde önemli etkilerde bulunmuştur. Tohumların içsel GA₃/ABA ve zeatin/IAA oranlarındaki azalışlar sırasıyla çimlenme oranının düşmesine ve hipokotil uzamasında yavaşlamaya neden olmuştur. Kontrol bitkilerin tohumlarına göre GA₃ uygulanmış bitkilerin tohumlarında kabuk oranı artmış, yağ içeriği ise azalmıştır. Gibberellik asit uygulamaları ile elde edilen hibrid aspir tohumlarında zayıf çimlenme ve çıkış gibi önemli problemler ile karşılaşılacağı sonucuna varılmıştır.

Anahtar Sözcükler: Aspir, *Carthamus tinctorius* L., Tohum fizyolojisi, Gibberellik asit, Bitki hormonları, Polen kısırlığı

Introduction

Safflower (*Carthamus tinctorius* L.) has been grown commercially as one of the world's oldest oilseed crops. India, USA, Mexico, Ethiopia and Argentina are the largest producers of safflower and these countries are responsible for over 95% of the world's safflower production (1). Safflower is primarily used for edible oil (rich in linoleic acid over 70%) as a salad and cooking oil, and for natural dyes (especially cartharmin) as a source of yellow and red dyes for clothing and food (2). Since safflower is more drought and salt tolerant than some other oilseed plants such as sunflower, it is especially

suitable for dry and salty areas where other oilseeds are difficult to grow (3).

However, safflower seed yield, as some 1000 kg/ha, is generally lower than that of other oil crops. For this reason, it is necessary to increase safflower yield via advanced plant breeding programs based on the development of hybrid varieties. The main aim of hybrid breeding is to benefit from heterosis occurring in the F₁ generation (4). Although safflower has great hybrid vigor in the F₁, it is not practically utilized because of the predominant self-pollination. The pollen sterility system is especially useful in hybrid breeding for obtained pollen

sterile female parents. For economical and practical hybrid seed production in safflower, attempts have been made to benefit from genetic or synthetic pollen sterility (5,6).

A plant hormone is an organic compound synthesized in one part of a plant and translocated to another part where, in very low concentrations, it causes physiological response and control (7). Physiological control in plants is governed by four classes of plant hormones: inhibitors such as abscisic acid that block germination; auxins that control root formation and growth; the gibberellins that regulate protein synthesis and stem elongation; and cytokinins that control organ differentiation (8). Gibberellic acid (GA_3) (actually a group of related substances called gibberellins) was discovered as a metabolic byproduct of the fungus *Gibberella fujikuroi*, which causes the stems of growing rice to elongate so rapidly the plant collapsed. GA_3 acid is a very potent hormone whose natural occurrence in plants controls their development. Although it has many effects regulating various physiological processes, including seed germination, the mobilization of endosperm storage reserves, shoot growth, flowering, floral development and fruit set, in recent years another important effect of GA_3 was found: it induced pollen sterility in some plants such as sunflower, rice and onion (9-13).

In addition, safflower plants treated with exogenous GA_3 applications to the buds before flowering produced no or insufficient pollen because of inhibition of microsporogenesis (6,14). This result was very important for practical hybrid seed production in safflower. However, it is also important to know how seeds from plants treated with gibberellic acid response to the regeneration. The objectives of this work were to measure the influence of GA_3 sprayed onto safflower plants on some physiological activity and endogenous hormone levels of the seeds.

Materials and Methods

The GA_3 treatments of a safflower (*Carthamus tinctorius* L.) cultivar namely 'Dinçer 5-118', which has a spineless capitulum and orange petals, were evaluated at the experimental field of Süleyman Demirel University, Faculty of Agriculture, Department of Field Crops in Isparta/Turkey. The seeds were sown on 1 April, 2000, at a recommended spacing of 45 by 20 cm in plots 3 x

1.3 m in size. Four different concentrations as 100, 200, 300 and 400 ppm (ppm = mg/liter) of GA_3 ($C_{19}H_{22}O_6$) were sprayed onto the buds before flowering 75 days after sowing for inducing pollen sterility (6). Non- GA_3 treated plants (controls) were sprayed with only pure water. After maturation, seeds were harvested from the plants treated with GA_3 . Hormone analysis was conducted on the seeds. For this purpose, firstly the endogenous hormones were totally extracted (15). Later, thin layer chromatography (TLC) was used for separation and purification of the hormone extracts (16). Finally, esterificated hormones were determined by capillary gas chromatographic (GC) analysis with a HRGC Mega 2 Type Fisions Model equipped with a flame-ionization detector (FID) and a Supelco SPB-1 (30 m x 0.32 mm, I.D.) column (17). The column temperature was held at 80 °C and the temperature was programmed to 280 °C at 5 °C/min. Other GC conditions were; injector, 200 °C; detector, 300 °C. Helium was used as carrier gas (22 psi). The levels of endogenous GA_3 , zeatin, indole-3-acetic acid (IAA) and abscisic acid (ABA) were determined as mg.g⁻¹ in seeds. Fifty seeds taken randomly from plants treated with GA_3 concentrations were germinated in a dark chamber at 25 °C in petri dishes with standard germination paper blotter moistened with tap water. At intervals of 24, 48, 72 and 96 h, the germination percentage (%) and hypocotyl length (mm) of the seeds in the petri dishes were measured. Some physical and chemical characters of the mature seeds such as seed weight (mg), hull percentage (%) and oil content (%) were evaluated. The oil content of the seeds was determined by nuclear magnetic resonance (NMR, New Port Analyser Magnet Type 10). The applications were conducted with four replications and the LSD test at the 5% level was used to determine significant differences among the treatments.

Results

The endogenous hormone levels of seeds from safflower plants treated with GA_3 for pollen sterility induction are presented in the Figure. The endogenous hormone levels of the seeds from treated plants were significantly different from those of the controls. Seeds from non- GA_3 treated plants contained GA_3 (57.2 mg.g⁻¹), zeatin (44.7 mg.g⁻¹), IAA (0.4 mg.g⁻¹) and ABA (0.6 mg.g⁻¹). In general, the levels of endogenous GA_3 and zeatin decreased, and the levels of the endogenous IAA

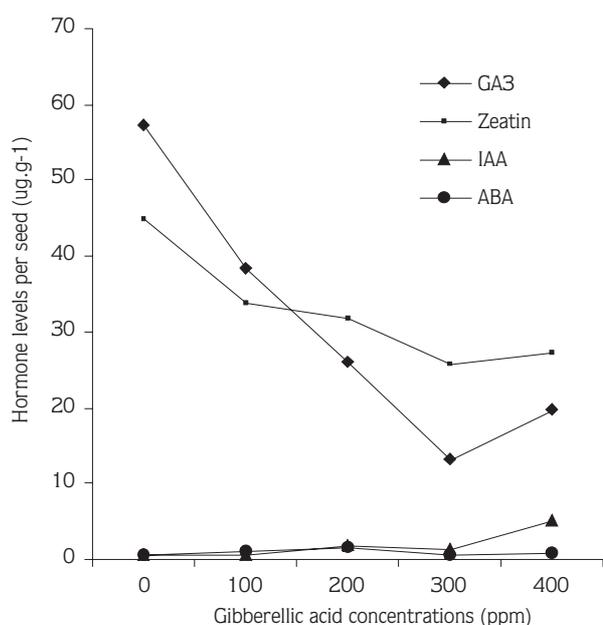


Figure. The endogenous hormone levels of seeds from safflower plants treated with gibberellic acid

and ABA increased with increasing exogenous GA₃ concentrations (Figure).

GA₃ and ABA, which are the two major endogenous plant growth regulators controlling seed activation, were changed in opposite directions in seeds treated with various exogenous GA₃ concentrations. Because gibberellins (GAs) and ABA act antagonistically in many aspects of seed development, it is important to evaluate the GA₃/ABA ratio. GA₃ application with concentrations of 0, 100, 200, 300 and 400 ppm caused an obvious decrease in the GA₃/ABA ratios in the seeds: 95.3, 42.5,

17.2, 26.0 and 28.0, respectively. Therefore, exogenous GA₃ application to plants raised endogenous ABA opposite to endogenous GA₃ in the seeds. In addition, because cytokinin (zeatin) and auxin (IAA) act antagonistically on shoot and root formation, it is important to evaluate the zeatin/IAA ratio. GA₃ treatments with concentrations of 0, 100, 200, 300 and 400 ppm also caused decreases in the zeatin/IAA ratios in seeds: 111.7, 56.3, 18.7, 21.4 and 5.4, respectively. Therefore, exogenous GA₃ application to plants raised endogenous IAA opposite to endogenous zeatin in the seeds.

The germination percentage and hypocotyl length of seeds produced from GA₃ treated plants are presented in Table 1. GA₃ treatment of safflower plants inhibited seed germination and hypocotyl elongation with increasing GA₃ concentrations, while seeds produced from non-GA₃ treated plants maintained viability. All treatments gave a decreased germination percentage and hypocotyl length compared to the controls. Inhibition of seed germination occurred particularly in the first 48 h period. During later periods, inhibition effects were less distinguished. However, decreases in the hypocotyl elongation of the seeds from GA₃ treated plants continued clearly up to the last period of 96 h (Table 1).

Some physical and chemical properties of seeds from plants treated with GA₃ are presented in Table 2. GA₃ treatments also significantly affected the physical and chemical characters of the seeds. Seed weight was reduced from 4.14 mg to 3.45 mg per seed, hull percentage was increased from 47.3% to 53.6%, and oil content was reduced from 30.6% to 24.1% by increasing exogenous GA₃ concentrations (Table 2).

Table 1. Germination percentage and hypocotyl length of seeds produced by plants treated with different gibberellic acid concentrations

Concentrations of GA ₃ (ppm)	Seed germination percentage (%)				Hypocotyl length (mm)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	90.0 a*	94.0 a	94.0 a	98.0 a	3.7 a	14.5 a	25.0 a	35.2 a
100	74.0 b	82.0 bc	84.0 b	86.0 b	3.5 ab	10.2 b	21.1 b	29.8 b
200	68.0 bc	84.0 b	90.0 ab	90.0 ab	3.3 b	7.3 bc	19.0 bc	26.8 b
300	48.5 c	77.1 c	85.7 b	85.7 b	3.2 bc	6.7 bc	16.3 c	23.0 bc
400	46.7 c	76.7 c	90.0 ab	90.0 ab	3.0 c	5.5 c	16.0 c	19.1 c

* Values within a group followed by the same letter or letters are not significantly different at the 5% level of LSD test.

Concentrations of GA ₃ (ppm)	Seed weight (mg per seed)	Hull percentage (%)	Oil content (%)
0	4.14 a*	47.3 c	30.6 a
100	3.40 c	49.5 bc	26.6 b
200	3.78 b	50.0 b	26.6 b
300	3.45 c	53.6 a	24.1 c
400	3.42 c	54.0 a	24.9 c

Table 2. Seed weight, hull percentage and oil content of seeds produced by plants treated with different gibberellic acid concentrations

* Values within a group followed by the same letter or letters are not significantly different at the 5% level of LSD test.

Discussion

It is known that gibberellins promote the germination of seeds in many species. In seeds, the principal gibberellin effect is to enhance cell elongation so the radicle can push through the endosperm, the seed coat that restricts its growth. However, ABA is a potent inhibitor of seed germination mainly because it slows radicle elongation and delays germination without preventing it (7,8,18). It was found in this study that exogenous GA₃ treatments of safflower plants inhibited endogenous gibberellic acid synthesis while they promoted endogenous ABA synthesis of seeds. The levels of endogenous GA₃ were gradually decreased in contrast to endogenous ABA levels increasing exogenous GA₃ concentrations, especially up to 300 ppm (Figure). The lowering endogenous GA₃/ABA ratio in the seeds decreased the rate of seed germination in particular (Table 1). Similar results were also reported in onion and rice (10,11). Since gibberellins and ABA act antagonistically in many aspects of seed development, it may be suggested that maturation phase gene expression is regulated by GA/ABA ratio in seeds. However, this suggestion needs to be studied further.

Application of GA₃ to plants inhibited hypocotyl elongation of the seeds (Table 1). This might be caused by a lowered zeatin/IAA ratio in the seeds due to GA₃ treatment. It is known that cytokinins and auxins have opposite effects on root-shoot formation. If the cytokinin-to-auxin ratio is lowered, young shoot (or

hypocotyl) elongation is restricted, and root formation is favored (7).

It was reported that hull percentage is negatively related to the kernel and oil content of the seeds (2,3). For this reason, improving the oil content of the seed by decreasing the hull percentage is one of the main aims in safflower breeding. GA₃ treatments of plants significantly affected these characters. The seeds from GA₃ treated plants had a lower weight, a higher hull percentage and a lower oil content than seeds from non-GA₃ treated plants (Table 2). Oil is the main energy source of developing seeds. For this reason, decreasing oil content in the seed kernels produced from GA₃ treated plants may also cause the seeds to have low germination viability and poor emergence vigor.

As a consequence, the poor germination and emergence vigor might be a major problem in hybrid safflower seeds produced from plants treated with GA₃. However, it must be discussed after more detailed research how much these negative results of GA₃ applications influence economical hybrid seed production and healthy hybrid plant development in safflower.

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