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Studies on the Onset of Germination, Seed Maturation and Development in Cucumber

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ABSTRACT: Study on seed development, the onset of germination, physiological maturity in cucumber (*Cucumis sativus* L) were undertaken to identify the suitable stage for seed extraction from the fleshy fruit. The manually pollinated fruits were harvested at periodic intervals, from 0-40 days, and the extracted seeds were tested for germination, dry matter accumulation, moisture content. The onset of germination at 12 DAP is far earlier than the physiological maturity. The decrease in germination during maturation drying was observed along with increased seed quality in seedling dry weight and seed vigour index. During various stages, the GA3 and ABA quantification during different seed developmental stages showed the relation between germination and the balance of these hormones. The germination initiated and increased with the increase in GA_3: ABA ratio. The high ABA content before the onset of seed germination enabled the seed to become dormant. Its slight increase during maturation drying for attaining stress tolerance during the loss of water was observed. In the present study, we noticed no relation between hormonal balance and seed vigour during developmental stages. The high vigour and germination above the Indian minimum seed certification standards at maturation drying made it a suitable seed extraction stage.

Keywords: ABA, GA_3, Physiological maturity, Seed vigour.

Cucumber (*Cucumis sativus* L) belongs to the family *Cucurbitaceae* and is indigenous to India [1]. Globally, cucumber is the fourth most important vegetable crop. In India, 1.70 million tonnes of cucumber produced every year from an area of 0.109 m ha, with average productivity of 15.60 t/ha [2]. Seed is the prime factor in modulating the quantitative and qualitative characteristics of a crop. Therefore, more attention to know the pattern of seed development, maturation, and germination before and during physiological and harvest maturity will help to produce quality seeds more effectively.

Seed development, from fertilization to the mature seed, can be divided into three phases, i.e., phase I (development of seed structures), phase II (accumulation of food reserves) and phase III (maturity) [3]. Seed developmental processes and environmental factors both play a crucial role during seed germination. Phytohormones (especially ABA and GA_3) are the signalling biomolecules that are responsible for seed germination. ABA is a sesqui-terpene hormone that is necessary during late embryogenesis to maintain the dormancy process. In contrast, GA is a di-terpene hormone regulating seed development, germination and other aspects of plant growth and development, such as leaf expansion, stem elongation and flowering. The expected interplay between the Reactive Oxygen Species (ROS) and growth hormones (ABA and GA_3) were reported to induce germination of seeds [4]. The balance between the two antagonistic hormones ABA and GA_3, and their crosstalk with ROS and other cellular signalling pathways decides whether an imbibed seed germinate or remains under pause [5-6]. Biosynthesis of ABA and GA begins in plastids by using a typical precursor, geranylgeranyl diphosphate (GGDP), which is synthesized along the 2-*C*-methyl-derythritol phosphate (MEP) pathway [7-9]. GA is a crucial hormone, which plays a major role during germination. Even so, some ABA-insensitive and ABA-deficient mutants can be germinated in the presence of paclobutrazol (PAC), an inhibitor of GA biosynthesis. An insignificant amount of GA is adequate for germination when ABA biosynthesis or signalling is hindered. These observations support the idea that the balance of endogenous levels of ABA and GA plays an essential role in controlling seed germination.

Identifying a suitable seed extraction stage from the fleshy fruits is vital to obtain good quality seed with high viability and vigour. Even though few studies were made on seed development and germinability of seed during development in cucumber, the vigour studies and role of hormones on germination initiation and vigour is still lacking. Thus, the present study was undertaken to identify a suitable stage for extraction of seed with maximum vigour and viability and to know the relevance of GA and ABA on these parameters.

MATERIALS AND METHODS

The studies were conducted in the Pusa Barkha cultivar of cucumber to identify germination initiation stage, physiological maturity, and harvest maturity. The cucumber plants were grown in the Indian Agricultural Research Institute's experimental field, New Delhi, in the sandy-loam soil at 90 × 45 cm spacing. Three flowers per vine were pollinated, covered with butter paper bags and tagged. Fruits were harvested at periodic intervals from 0 to 40 days, and seeds were extracted manually. The seeds were obtained from the harvested fruits at different growth stages and tested for physiological and biochemical parameters.

Determination of seed moisture content

The seed moisture content was determined on a weight basis as per ISTA rules [10]. Briefly, three replicates of 10 seeds each were weighed fresh and dried at 101°C for 17 hrs. Based on the weight difference, the per cent moisture content was calculated over a fresh weight.

Germination percentage

The onset of seed germination was tested following ISTA rules [10] for standard germination test with some modification. To break the dormancy, seeds were soaked in 100 ppm GA_3 solution for 17 h, and after that, the surface washed with distilled water thoroughly and proceeded for seed germination. Three replications of 10 seeds were placed on pre-soaked filter paper in Petri plates and kept in a germination chamber at 25°C for 14 days. Seeds with radicle protrusion of more than 2 mm size were considered as germinated. The per cent germination calculated based on the number of seeds germinated and the total number of seeds kept for germination

Seed and seedling dry weight

The dry weight of the seeds was measured using the constant high temperature (101°C) oven method. Ten seeds in three replications were dried for 17 hours to record the dry weight. The seedling dry weight was measured in three replications at the end of the germination period by exposing them to a constant temperature of 80ºC for 24 hours.

Vigour Index

The seed vigour index was estimated as per Abdul Baki and Anderson [11]. Briefly, the germination percentage of the seeds was multiplied with the dry seedling weight in grams to obtain the unit less vigour index.

Quantification of growth regulators

The two key growth regulators controlling seed germination/dormancy, i.e., GA_3 and ABA, were estimated using an Agilent 1100 series High-Performance Liquid Chromatography (HPLC) as per the protocol of Kelen et al. [12]. Briefly, the separation was accomplished using 4.6 mm x 250 mm, stainless steel Agilent zorbax eclipse XDB C-18 analytical column with 5 µm particle size. The mobile phase used throughout the study was acetonitrile: water (26:74, v/v). Phosphoric acid (0.03M) and NaOH were used to set the pH 4.0 of the mobile phase. The column temperature was maintained at 30ºC, and separation was done with a flow rate of 0.8ml/min. The injection volume of 20µl was followed in all samples. To compute each component's retention time, a working sample of known concentration from a stock solution was analyzed separately. The retention time t_R is the time from the sample injection to compound elution, taken at the maximum (apex) of the peak that belongs to the specific molecular species (known or not). The t_R for GA_3 is 6.606 min (Figure 1a), and ABA is 23.231 min (Figure 1b) when the absorbance was measured at 206 and 265nm, respectively.

RESULTS AND DISCUSSION

The seeds were tested for germination at two days interval, from 0-40 days after pollination (DAP). Seeds were not able to germinate till 10 DAP. The first germination was observed at 12 DAP, and 60 per cent germination was recorded (Figure 2). Further, the germination sharply increased after 16 DAP (60%) till 24 DAP (80%) and then decreased and got stabilized at 70% till 40 DAP. A similar kind of result was noticed in MTi 2 cultivar of cucumber [13], where the germination was maximum at 25 days from flowering and afterwards got reduced. They observed a similar pattern of germination

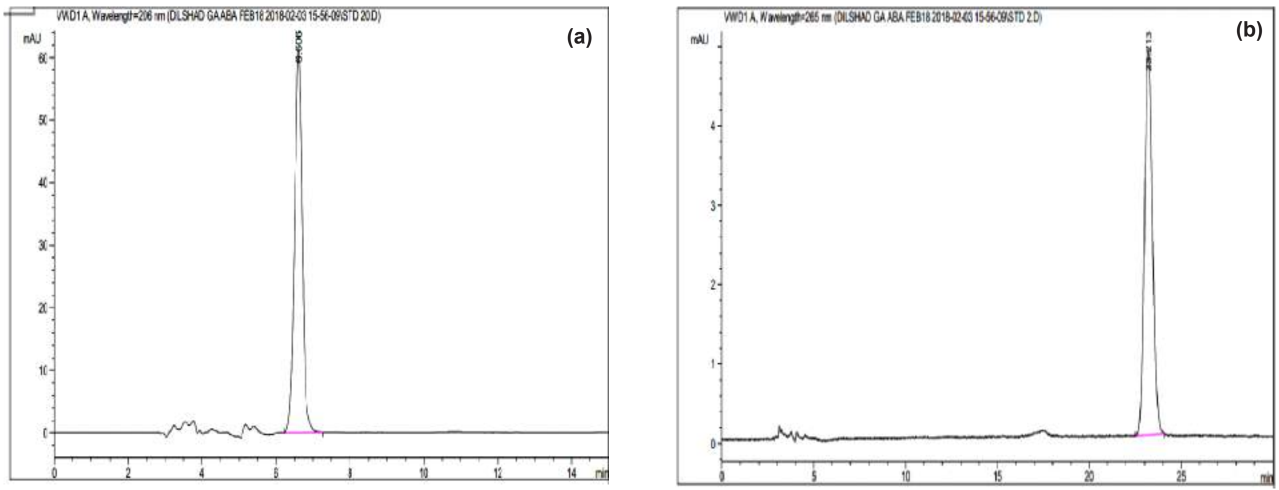

Figure 1. Chromatograph showing retention time of growth regulators (a) GA_3 (b) ABA

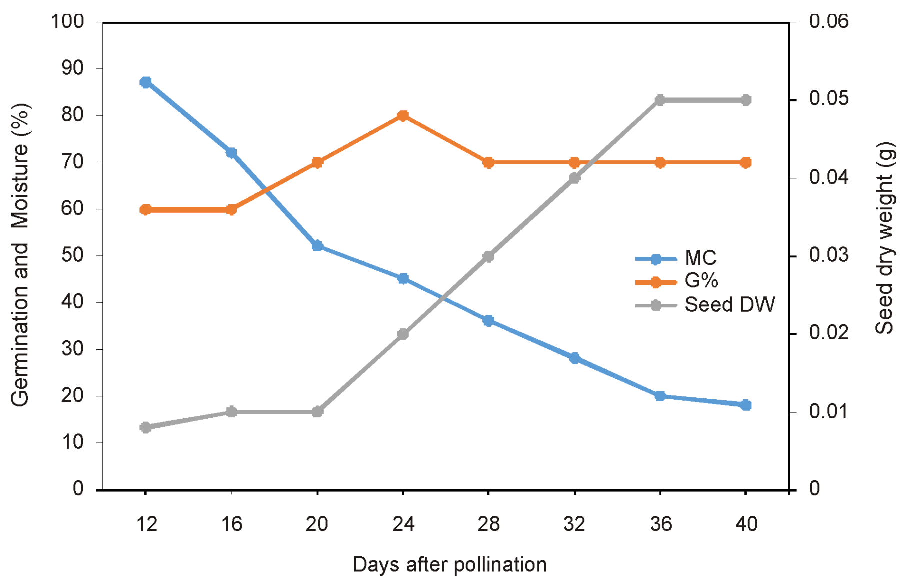

Figure 2. Germination, moisture content and seed dry-weight at different stages of seed development

both in dried and fresh seeds of the cucumber. They interpreted that dormancy induction may cause a decrease in germination in the later stages of seed maturation. The seed quality in terms of germination and vigour will increase with the fruit ripening and is maximum when fruits reach edible maturity stage and later will decrease in overripe fruits [3]. In cucumber, the germinability is acquired even before the desiccation tolerance achieved [13].

The seed's moisture content gradually decreased from 87% on a fresh weight basis at 12 DAP to 20% on 36 DAP. Afterwards, the decline was marginal till 40 DAP. The decline in moisture content coincided with the increase in seed dry weight. The maximum dry weight was observed at 36 DAP and then got stabilized (Figure 2). The maximum dry weight is an indicator of the physiological maturity stage for the seed. Physiological maturity in seeds is to recapture the reproducing capacity; it usually coincides with maximum dry weight attainment [3]. During which the flow of nutrients from the mother plant to the seed freezes [14]. Similar kinds of results were obtained by [13] in cucumber, where they found the maximum dry weight at 33 days from flowering, which coincided with moisture loss. In the present study, the moisture content dropped sharply by 70%, coinciding with the maximum dry weight, indicating that increased dry matter replaced the moisture during seed maturation. Seed maturation is the last phase of seed development during which the seed undergoes physiological, biochemical and morphological changes [15]. However, seed development variations depend on the crop species and the seed crop's biogeographic distribution [16]. The loss of seed moisture content during maturation is an essential/integral part of seed development [17-18]. Fresh weight, dry weight, and moisture content in developing seeds varied for seed development stages, and these differences varied in genotypes in different magnitudes. The significant decline in moisture content as the seed maturity advances is correlated with a higher dehydration rate of seeds in many small-seeded *Cucumis* spp. [19-20]. In the present study, it was observed that the cucumber seed attained maximum germination (24 DAP) even before reaching its physiological maturity (36 DAP), as identified in terms of maximum dry weight (Figure 2). The maximum germination may coincide with the physiological maturity or maturation drying after attaining physiological maturity in several crop species. However, in fleshy fruits where the seeds are embedded in the fruit, it will coincide with the fruit maturity as the surrounding fleshy tissue has high moisture [3]. The present study also supported the same finding where the fruit is suitably ripened at 24 DAP and is almost dried up at 36 DAP.

To know the hormonal influence, the two crucial hormones controlling germination, GA_3 and ABA, were quantified at four different stages of seed development viz, before the onset of germination (10 DAP), the onset of germination (12 DAP), maximum germination (22 DAP) and post physiological maturity (38 DAP). The highest GA_3 content (30.35 µg/g fw) was observed in seeds coinciding with maximum germination (22 DFP), and the lowest GA_3 content (11.89 µg/g fw) was recorded after attaining physiological maturity (Figure 3). The GA_3 content increased during seed development up to the maximum germination stage and then decreased during the maturation drying phase (Figure 3). The seed germination showed a high positive correlation with the GA_3 content during different developmental stages (Table 2). The ABA content was highest at 10 DAP (4.62 µg/g fw) and gradually decreased with the progress of seed development (Figure 4). The minimum ABA content (2.230 µg/g fw) coincided with the maximum germination stage, and later there was an increase in ABA content at

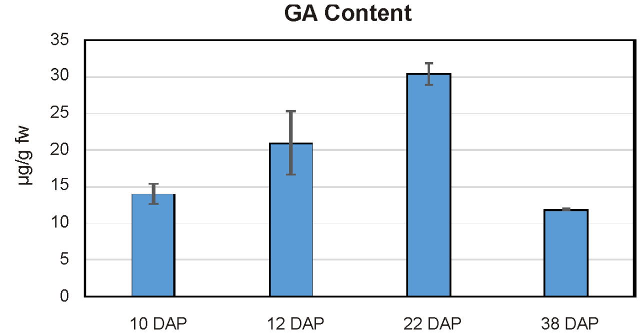

Fig. 3. GA_3 content during different stages of seed development

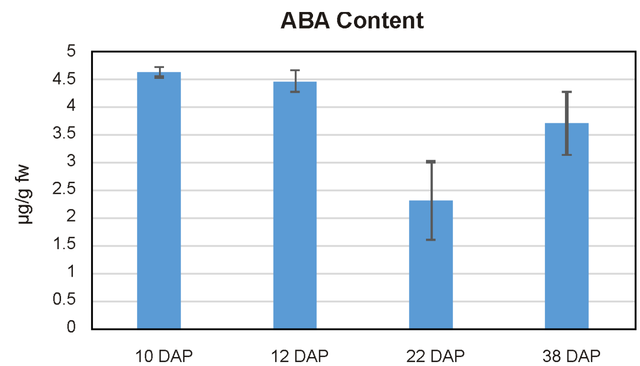

Figure 4. ABA content during different stages of seed development

around physiological maturity. The pattern of ABA content during seed development showed an inverse relationship with germination. A negative correlation was noticed between ABA and germination during seed development (Table 2).

Similarly, an antagonistic effect was observed between ABA and GA_3 content during different stages of seed development. The change in GA/ABA balance, rather than absolute hormone amounts, controls germination [21]. In the present study also, a high ratio of GA_3 and ABA was observed with the germination induction and increase during seed development (Table 1). The maximum ABA content before the onset of seed germination and a later slight increase during harvest maturity confirms its role with early germination inhibition and decreased germination during maturation drying, respectively. During the first phase of seed development, ABA is produced in maternal tissues and subsequently in the embryo, where it accumulates during the mid-phase of seed development after that slightly reduced. Although required to complete germination, gibberellic acid is not directly involved in many processes during germination, such as the initial mobilization of seed storage proteins

Table 1. Quantification of GA_3 and ABA during different stages of seed development

DAP	GA_3/ABA	Germination (%)	Seedling dry weight (g)	Vigour index
10	3.03	0	0	0
12	4.69	60	0.01	0.60
22	13.18	80	0.02	1.60
38	3.20	70	0.06	4.20
LSD ($p \leq 0.05$)	2.81	3.99	0.003	0.29

Table 2. Correlation between hormones and seed quality parameters during seed development

	GA_3	ABA	GA_3/ABA	Germination	Seedling dry weight	Vigour Index
GA_3	1					
ABA	-.725	1				
GA_3/ABA	.943	-.901	1			
Germination	.517	-.709	.566	1		
Seedling Dry weight	-.316	-.315	-.104	.591	1	
Vigour Index	-.269	-.374	-.045	.610	0.998	1

and lipids, which may be the reason for its decrease during maturation drying. The ABA plays a crucial role in imparting abiotic stress resistance [22], and during maturation drying, the increased content of ABA in the seed helps in tolerating the loss of water.

The seedling dry weight and vigour index have shown a gradual increase during seed development (Table 1). The seedling dry weight increased from 0.01 mg to 0.06 mg from 12 DAP to 38 DAP. Similarly, a 600 per cent increase in vigour index was observed during the same period (0.6 to 4.2). However, no relationship was observed between hormonal content and these two vigour parameters. The seedling dry weight and vigour index being the post germinative traits, might be less influenced by the GA_3 and ABA interaction which is more crucial for germination per se. The high vigour during the maturation drying phase is also an indicator of the seed's better longevity. Thus, the maturation drying phase, i.e., at 38 DAP under Delhi conditions, is a better seed harvesting stage than 22-24 DAP, where high germination was recorded.

CONCLUSION

Identifying the seed extraction stage in cucumber is essential to have a high quality of seed for sowing purpose. During seed development in the fleshy fruit, the germination ability is obtained at 12 DAP, i.e., long before attaining physiological maturity (36 DAP). Even though germination is highest at 24 DAP coinciding with the fruit ripening and high GA_3: ABA ratio, the seed quality in terms of vigour is highest at the maturation drying stage, which is 38 DAP under Delhi conditions. Even though germination was reduced to 70% at this stage, it is still far above the Indian minimum seed certification standard (60%). Thus, seed extraction at the maturation drying stage in cucumber is recommended for obtaining high-quality seed.

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