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Seed Development and Maturation in Ashwagandha (*Withania somnifera* Dunal.)

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ABSTRACT: Studies were undertaken at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, on Ashwagandha (*Withania somnifera* Dunal) var. Jawahar Asgand 20, to trace the pattern of seed development and maturation. The seeds attained physiological maturity at 35 DAA when seed dry weight was at maximum and it was also associated with the colour change of the fruit from orange to deep red. The developing seeds attained the germination potential of 21 per cent at 21 DAA and reached the maximum of 63.5 per cent at 42 DAA. The root and shoot length of seedlings, dry matter content and vigour index values increased gradually as the seed reached maturation. Electrical conductivity of seed leachate, enzyme activities such as α-amylase, catalase and peroxidase decreased gradually during seed development and maturation. Harvest maturity of seed was reached at 42 DAA when seed germination and vigour were at peak. High moisture content of fruits and seeds at initial stages got reduced to 60.6 and 32.3 per cent respectively at 42 DAA.

Key words: Ashwagandha, Seed development and Maturation.

Ashwagandha (*Withania somnifera* Dunal) is one of the important medicinal plants being cultivated in India and is commonly known as 'Winter Cherry'. Ashwagandha is mentioned as an important drug in ancient Ayurvedic literature. The alkaloids such as 'withanine' and 'somniferine' present in roots of ashwagandha posses pharmacological activity [1]. Roots of ashwagandha are prescribed in medicines for hiccup, aphrodisiac, seminal debility, several female health disorders, bronchitis, rheumatism, dropsy and skin diseases [2]. Extensive information is available on cultivation and agronomic practices [3], genetic variability [2], alkaloidal properties [4] and physiology[5] of aswagandha. However, information available on seed quality is very limited. Moreover, though seed is the primary propagating material in this crop, the germination potential of the seeds is very low

A systemic study on tracing the pattern of seed development and maturation is highly essential to obtain seeds with good quality characteristics. To gain an insight into the source-sink relationship and to manipulate the agronomic practices, the knowledge on morphological, physiological and biochemical changes occurring in the developing seed assumes significance. Seed maturation refers to the morphological, physiological and functional changes that occur from the time of fertilization until the mature seed is ready for harvesting [6]. To reduce the risks of various biotic and abiotic hazards and the possible loss of seed quality, harvesting the seeds at their physiological maturity stage is recommended [7]. A number of parameters such as moisture content, fresh weight of seed [8], dry weight of seed [6], protein content [9] etc., have been used in several studies to assess the physiological maturity.

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MATERIALS AND METHODS

Seed crop of *W. somnifera* was raised during March, 1999, at Tamil Nadu Agricultural University farm, Coimbatore, in four beds of size 3 x 2 m^2 to form four replications. About 250 flowers were tagged at the time of anthesis in each replication. Sufficient number of fruits were collected at seven days interval from the date of tagging *viz.*, 7, 14, 21, 28, 35 and 42 days after anthesis (DAA). The following observations were recorded at each stage of maturation.

Fresh weight of fruit: Immediately after collection, the fruits were weighed individually and the mean weight was expressed in mg $fruit^{-1}$.

Dry weight of fruit: After determining the fresh weight, the same fruits were dried in shade initially and then in a hot air oven maintained at 105 ± 2°C for 16 h. After drying, the fruits were cooled in a desiccator for 30 minutes and the dry weight was recorded and expressed in mg $fruit^{-1}$.

Moisture content of fruit [10]: Four replicates of ten fruits each were used for determining the moisture content using low constant temperature oven method and was expressed as per cent using the following formula:

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where,

M_1 - weight of the moisture bottle alone

M_2 - weight of the moisture bottle + sample before drying

M_3 - weight of the moisture bottle + sample after drying

Fresh weight of seed: Seeds were extracted from four replicates of 10 fruits each and the fresh weight of seeds was recorded and the mean expressed in mg $seed^{-1}$.

Dry weight of seed: The seeds used for fresh weight determination were dried initially in shade and then in a hot-air oven maintained at 105 ± 2°C for 16 h. After drying, the seeds were cooled in a desiccator for 30 min. The dry weight of seeds was recorded and the mean expressed in mg $seed^{-1}$.

Moisture content of seed [10]: The moisture content of seeds was determined following the procedure as detailed for moisture content of fruit.

Germination percentage [10]: Four replicates of 100 seeds were placed on top of paper and kept in a germinator maintained at alternating temperature of 25 & 30°C (16 hours and 8 hours, respectively) and 95 ± 2 per cent RH. After 17 days, the seedlings were evaluated and the normal seedlings were counted and expressed in percentage.

Root length [8]: Ten normal seedlings from each replication of germination test were taken at random and the length between the collar and tip of primary root was measured and the mean value was expressed in cm.

Shoot length [8]: The same seedlings used for root length measurement were used for shoot length measurement, by measuring from collar to tip of primary leaves and the mean value was expressed in cm.

Dry matter production [8]: The seedlings used for growth measurements were initially sun dried and then kept in a hot air oven maintained at 85°C for 24 h. Then the seedlings were cooled in a desiccator for 30 min and the weight was recorded.

Conversion efficiency index (CEI): It was calculated using the following formula and expressed as whole number [11]

$$\text{CEI} = \frac{\text{Seedling dry matter content}}{\text{Dry weight of seed}} \times \text{Germination \%}$$

Electrical conductivity: Four replicates of 250 mg of seeds were surface sterilised with 0.1 per cent mercuric chloride solution and rinsed with distilled water. To facilitate complete soaking, the seeds were tied in a nylon mesh along with glass beads and soaked in 30 ml of distilled water for five hours at 20°C. The electrical conductivity of the seed leachate was measured with digital conductivity meter with a cell constant of one and expressed as dSm^{-1} [12].

Protein Content [13]: The protein content of seeds was estimated by the colorimetric method. For each treatment 100 mg of powdered seed material was taken in a 50 ml polyethylene screw cap bottle and 25 ml of 1 N NaOH was added. The mixture was shaken for 30 min on a wrist action shaker to disperse the protein. Then, 10 ml of the suspension was poured into a graduated test tube and used as blank to compensate for the differences in the amount of natural pigments extracted. To the remaining suspension in the bottle, 0.25 ml of 10 per cent copper sulphate was added and the bottle was shaken for five minutes to develop the colour complex. The sample solution was then poured into a separate test tube and was left over night to allow the dispersed material to settle down. After centrifugation for 10 min, the optical density of the clear supernatant solution was measured in a spectrophotometer at 620 nm, along with suitable blank. From mean optical density value, the percentage protein for each treatment was calculated as follows:

Protein content = 3.78 + (61.6 x OD Value)

-Amylase activity [14] : Seed samples of 250 mg each were homogenized in 1.8 ml of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuged at 2000 rpm for 10 min.

The reaction mixture consisted of one ml of 0.067 per cent starch solution and 0.1 ml of enzyme extract. The reaction was stopped after 10 min of incubation at 25°C by the addition of one ml iodine HCl solution which contained 60 mg KI and six mg I_2 in 100 ml of 0.05 N HCl. Change in the colour was measured at 620 nm. The activity was calculated and expressed as mg of maltose g^{-1} of seed min^{-1}.

Catalase activity [15]: Seed samples of 250 mg each were homogenized in 0.066 M sodium phosphate buffer (pH 6.8) and centrifuged at 2000 rpm for 10 min. The reaction mixture consisted of 5 ml of phosphate buffer (pH 6.8), 4 ml of 0.3 N hydrogen peroxide (substrate) and 0.2 ml of enzyme extract. The reaction was stopped after 15 min of incubation, by addition of 10 ml of 2N H_2SO_4. The blank was maintained for each set in which 0.2 ml of enzyme extract was added after the addition of 2N H_2SO_4. The contents were titrated against 0.1 N $KMnO_4$ and difference between the titre values were estimated as the volume of permanganate equivalent to enzyme activity. The activity was expressed as μg of H_2O_2 mg^{-1} of seed min^{-1}.

Peroxidase activity [16]: Seeds samples of 250 mg each were homogenized in 5 ml of 0.25 M Tris buffer (pH 6.0) and centrifuged at 10,000 rpm for 10 min at 5°C to extract enzymes. 0.4 ml of enzyme extract, 0.5 ml of one per cent H_2O_2 and 0.5 ml of 0.5 per cent aqueous solution of pyrogallol were added and incubated for 10 min at 25°C.

The reaction was stopped by adding 0.5 ml of 5 per cent H_2SO_4. The OD at zero time and at the expiry of 10 min was measured in spectrophotometer at 420 nm. The peroxidase activity was expressed as difference in OD 10 min^{-1}.

RESULTS AND DISCUSSION

The fresh and dry weight of fruit increased progressively from the day of anthesis to 35 DAA and thereafter decreased. A steep rise in fruit fresh weight and dry weight was observed between seven and 14 DAA. At this stage of development, the fresh weight of fruit increased from 30.3 to 90.5 mg $fruit^{-1}$ and dry weight of fruit increased from 3.6 to 20.2 mg $fruit^{-1}$ (Table 1). The rapid increase at initial stages might be due to more uptake of water when the intake of carbon and nitrogen was comparatively low [17]. The decrease in fresh weight of fruit after 35 DAA was associated with dehydration of maturing fruits. The reduction in dry weight of fruit after 35 DAA might be due to oxidation and volatilization of chemical entities [18].

The fruit and seed moisture contents recorded at seven days after anthesis were 88.1 and 81.5 per cent, which decreased subsequently and reached the minimum of 60.6 and 32.3 per cent, respectively (Table 1) at 42 DAA. The quantum of moisture reduction during the developmental period was remarkably greater in seed than in fruit. This was in conformity with the findings

Table 1. Physical properties of fruit and seed during development and maturation in ashwagandha

Days after anthesis	Fruit			Seed		
	Fresh weight (mg fruit^{-1})	Dry weight (mg fruit^{-1})	Moisture content (%)	Fresh weight (mg 10 seeds^{-1})	Dry weight (mg 10 seeds^{-1})	Moisture content (%)
7	30.3	3.6	88.1 (60.8)	1.8	0.3	81.5 (64.5)
14	90.5	20.2	77.6 (61.6)	3.4	0.6	76.2 (60.8)
21	114.3	32.6	71.4 (57.6)	4.7	1.3	51.2 (45.6)
28	121.3	42.3	65.1 (53.7)	5.2	1.7	41.5 (40.1)
35	132.0	49.2	62.7 (52.3)	5.6	2.3	36.8 (37.3)
42	103.5	40.7	60.6 (51.1)	4.3	2.1	32.3 (34.6)
SEd	5.0	3.73	8.98	0.22	0.14	0.57
CD (P=0.05)	10.8	8.13	19.5	0.49	0.29	1.24

(Figures in parentheses indicate arcsine values)

in *Cassia angustifolia, Catharanthus roseus* and *Hibiscus sabdarrifa* [19]. Decline in the moisture content might be associated with the deposition of reserve materials as revealed by the increase in dry weight throughout the developing period [20].

The fresh and dry weight of seed attained maximum values at 35 DAA, recording 5.6 and 2.3 mg 10 seeds^{-1} respectively and decreased thereafter. Physiological maturity is normally defined as the stage at which the seed attains its maximum dry weight [18]. Once the seed attains maximum dry weight, the vascular connection between fruit and mother plant is severed [21] and stoppage of translocation of nutrients from mother plant to the fruit occurs. Some nutrients from fruit might also be translocated to the seed along with elimination of moisture during desiccation of fruit, whereas the loss in fresh weight of seed might only be due to the loss of moisture eliminated through dehydration and desiccation [6].

Among the physiological manifestations of the seed, germination played a significant role. The developing seed attained 22.5 per cent germination at 21 DAA (Table 2). It was reported to be 25 DAA in *Solanum ptycanthum* [22].

Even though germination ability was initiated in the early stages of seed development, it was increased with increase in maturity of seed. Maximum germination of 63.5 per cent was reached at 42 DAA (Table 2). The reason for nil germination, registered up to 14 DAA might be due to immaturity of the embryo. In the present study, there was 2.5 per cent increase in germination after 35 DAA. It was reported [23] that this increment might be due to the dehydration process, which might have played a facilitating role for germination.

The relative length of root and shoot of seedlings would predict their subsequent growth and performance [24]. In the present study, the root and shoot length of seedlings attained the maximum at 35 DAA, recording 2.85 and 2.93 cm respectively, which coincided with the maximum accumulation of fresh and dry weight of fruits and seeds. After 35 DAA, contrary to germination, there was a reduction in root and shoot length, reaching 2.78 and 2.88 cm respectively, at 42 DAA. This result was in conformity with [23] reports on cauliflower seedling performance.

Table 2. Physiological properties of seed during development and maturation in ashwagandha

Days after anthesis	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour index	Conversion efficiency index	Electrical conductivity (dSm^{-1})
7	0.0 (3.39)	-	-	-	-	-	0.980
14	0.0 (3.39)	-	-	-	-	-	0.873
21	22.5 (28.3)	1.82	1.90	9.6	84	164	0.435
28	55.3 (48.0)	2.65	2.71	15.5	296	504	0.216
35	61.0 (51.5)	2.85	2.93	19.3	329	525	0.103
42	63.5 (52.8)	2.78	2.88	18.5	354	580	0.069
SEd	1.45	1.12	0.01	0.22	3.05	3.01	0.003
CD (P=0.05)	3.16	0.26	0.04	0.47	6.65	6.57	0.073

(Figures in parentheses indicate arcsine values)

Dry matter content of seedlings could be taken as the manifestation of the physiological efficiency, dependent on seed vigour [25]. There was quantum increase in dry matter content towards the attainment of physiological maturity. The seed dry weight and dry matter content of seedlings are corollary and is an established fact.

Vigour index and conversion efficiency index increased as that of germination till 42 DAA. Developing seeds may record maximum germination even before attaining fullest maturity [26], but the vigour potential would reach the highest only at the fullest maturity [27]. In the present study, the developing seed attained maximum dry weight at 35 DAA, but the vigour index as estimated through germination and seedling growth, attained maximum value at 42 DAA. This is due to the increase in germination percentage after the maximum dry matter accumulation period of 35 DAA. Therefore, apparently, in developing seed, an in built mechanism is developed to withstand post-harvest operations like drying, after reaching the physiological maturity when changes in cell membrane and disorganisation of cell organelles takes place [28 & 29].

The maximum electrical conductivity of seed leachate (0.980 dSm^{-1}) was registered at seven days after anthesis (Table 2). As maturity advanced, it decreased and reached the minimum value (0.069 dSm^{-1}) at 42 DAA, indicating that membrane integrity was maximum at this stage. More electrical conductivity recorded at earlier stages might be due to poor integrity of cell membrane and immature nature of embryos. Similar results were reported in marigold [31] and in amaranthus [32].

Protein bodies which are subcellular structures commonly found in the cells of seeds, provide the compartment in which storage proteins are deposited during seed development [32]. It has been reported that seed dry weight, nucleic acid, lipid and protein accumulation followed almost a sigmoidal pattern in developing seeds while DNA and RNA accumulation slowed down earlier [33]. In the present investigation, the rate of accumulation of protein was much higher during early stages of development which was in conformity with [34]. The protein accumulation increased gradually and reached maximum at 35 DAA. Similar trend was recorded in developing seeds of *Hibiscus sabdariffa* [35].

Table 3. Biochemical properties of seed during development and maturation in ashwagandha

Days after anthesis	Protein content (%)	α-Amylase activity (mg of maltose g^{-1} of seed min^{-1})	Catalase activity (μg of H_2O_2 g^{-1} of seed min^{-1})	Peroxidase activity (OD 10 min^{-1})
7	7.35	1.79	3.96	0.072
14	9.40	1.75	3.92	0.080
21	11.53	1.66	3.63	0.082
28	11.83	1.62	3.42	0.078
35	12.10	1.59	3.14	0.075
42	11.42	1.57	2.99	0.070
SEd	0.04	0.02	0.02	0.004
CD (P=0.05)	0.08	0.05	0.04	0.007

The highest activities of a-amylase and catalase occurred at seven days after anthesis in the developing seed which coincided with the highest moisture level. Thereafter, the activities of both the enzymes reduced and reached the lowest level at 42 DAA. The activity of a-amylase was induced by the activity of gibberellins, and the same was synthesised and maintained till hydrated stage of seed development. Thereafter, the action of gibberellins reduced and thereby, it could not induce the activity of a-amylase as maturation advanced [36]. According to [37], the catalase is an oxidase enzyme and is a heamo protein appearing in free form and not bound with membrane. In seed development, the catalase activity reduced and attained minimum at maturity.

From the present study, it could be concluded that the ashwagandha seed reached physiological maturity at 35 DAA when the dry weight of seed was maximum [18] and it was associated with the colour change from orange to deep red. Harvest maturity was attained at 42 DAA when the seed germination and vigour potential was at peak.

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