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RESEARCH PAPER

Differential Seed Germination Responses of Ashwagandha Seed Collected at Different Time Period

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ABSTRACT

Ashwagandha, a medicinal plant, seed are matured at different level and its moisture contents are differed at various harvesting time. Resulting poor seed germination and have inferior seed quality. The present investigation was framed to enhance seed quality with higher seed germination of freshly harvested seed without any treatments in natural condition by evaluating the best harvesting time of seeds. Seed collection was start from January and ended in April; fresh and dry weight of 1000 seed with its moisture content at the time of harvest was differed. A significant result was showed in terms of seed germination percentage and other seed quality parameters recorded at the time of seed germination. Maximum seed germination rate of freshly harvested seed was observed in those seed harvested in 10th March (56.48%) with significantly higher root length (0.73 cm), germination energy (3.08), germination index (0.90), vigour index I (185.88) and II (18.35) with minimum days to germination completion (18.33 days). Therefore, seed harvested during the month of March to April showed better seed quality as compared to January and February month.

HIGHLIGHTS

- Ashwagandha seed are matured at different time period.
- Matured harvested seed contents different level of moisture and dry weight.
- Significantly higher germination percentage and other seed quality parameters was observed in seed harvested during March month.
- Increase the germination percentage in freshly harvested seed of more than 50%.

Keywords: Harvesting period, seed moisture, germination, vigour index I and II

Ashwagandha, *Withania somnifera* belongs to the family Solanaceae commonly known as "Indian Ginseng" or "Indian Winter Cherry" holds very prominent and potential place in traditional system of medicine in India (Ayurveda). In India it is mostly cultivated in Madhya Pradesh, Gujarat, Rajasthan, Maharastra, Punjab, Haryana and Uttar Pradesh state. The crop is grown mostly in semi-tropical areas receiving rainfall of 500 to 750 mm as rainfed crop. The root, stem and leaf are use in various treatment of diseases. The best economic part of this plant is considered as root; use as vital tonic, anti-stress, controlling of depression, skin diseases, ulcers and anti-arthritic, anti-oxidant and anti-tumor agent. The major bioactive compounds are alkaloid and steroidal lactones (withanolides and withaferins)and saponin (Mishra *et al*. 2000). The crop is huge potential at national and international market commercially (Kothari *et al*. 2003) but due to over exploitation in an unregulated manner the medicinal plants are exhausting day by day.

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The crop is generally sown during the 1st fortnight of September and starts flowering and fruiting in the month of December and finally ready to harvest at 150 to 180 days after sowing (DAS) in the month of February to April. The leaves turn dry and orange-red berries along with calyx turns into brown indicate that maturity of berries. The crop is harvested by uprooting entire plant followed by root are separated by cutting the stem 1-2 cm above the crown and berries are collected separately. The mature berries contain number of seed and use for propagating material. However, seed are matured unevenly on account of flowering is not synchronized in ashwagandha plant. Therefore, seed collected at the time of last uprooting time perhaps it contains different level of moisture which will greatly impact on germination rate and vigour of the seed. In fully mature berries and seeds content moisture of around 37 and 14 % respectively (Khanna *et al*. 2013). The size of seed, weight, texture and moisture content (MC) are the most important factors affecting seed germinability and longevity (Zheng *et al*. 1998). Seed quality is affected by various factors including harvesting time (Emilly *et al*. 2016). Three stages of maturity namely physiological, mass and harvest maturity are closely linked to regulate the seed quality with respect to the time of harvesting. Normally ashwagandha seed showed poor in germination and high mortality rate of seedlings under field condition (Vakeswaran and Krishnasamy, 2003).In previous finding it was revealed that enhancement of ashwagandhaseed germination through the use of plant growth regulator (GA_3) (Vakeswaran and Krishnasamy, 2003) and providing optimum temperature and light (Kambizi *et al*. 2006). Propagation by natural re-seeding is thus no longer sufficient to guarantee the survival of this plant. Considering all these points, it is very much necessary to outbreak this constraint in cultivation practices of ashwagandha. On this context, present investigation was framed to find out the best harvesting time of seed for better seed germination and vigour naturally.

MATERIALS AND METHODS

The completely brown matured berries of *Withania somnifera* variety Guajrat Anand Ashwagandha 1 (GAA 1) were collected from Medicinal and Aromatic Plants Research Station farm, AAU, Anand at every 10 days interval starting from January to April, 2023. Therefore, there were total 12 different date of harvesting and is mention in Table 1.

Table 1: Date of ashwagandha seed harvested from the field condition

Sl. No.	Date of harvesting	Sl. No.	Date of harvesting
1	T1: 10th January	7	T7: 10th March
2	T2: 20th January	8	T8: 20th march
3	T3: 30th January	9	T9: 30th march
4	T4: 10th February	10	T10: 10th April
5	T5: 20th February	11	T11: 20th April
6	T6: 28th February	12	T12: 30th April

Thereafter, seeds were collected from harvested berries. From the harvested seed lot fresh 1000 seed weight was recorded. To determine the dry weight and moisture content, seeds were kept in hot air oven at 100± 2°C (Ramashia *et al*. 2018). The dry weight of 1000 seed was measured and moisture content (fresh weight basis) was calculated by using given formula;

$$\text{Moisture (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where,

$W1$ = Empty cup weight

$W2$ = Cup and seed sample weight before drying

$W3$ = Cup and seed sample weight after drying

For seed germination test from each harvested seed lots, seed were air dried at normal room temperature. Completely dried uniform healthy seed was selected for germination studies. After that seed germination was checked by keeping in seed germinator. For these 50 seed in three repetitions were kept in petri dish lined with germination paper under the seed germinator with the maintained of temperature 28± 2°C and relative humidity 80± 2% in continuous light condition. The germination paper was moistened using distilled water whenever its necessary. Those seed that attained plumule length of 0.5 cm was considered as germinated seed. Germination percentage (GP) was calculated as per the ISTA, 2015 and days to germination completion (DGC) was recorded by considering last seed showed germinated.

Germination Percentage (GP) =

$$\frac{\text{No. of germinated seed}}{\text{Total no. of seed sown}} \times 100$$

Germination energy (GE) provides the information of germination rate and consistency which related to the seed vitality (Gupta *et al*. 2022). It is calculated as per formula given by Maguire 1962.

Germination Energy (GE) =

$$\frac{\text{Germination Percetnage}}{\text{Days to germination completion}}$$

Germination index (GI) measures the germination and speed of germination (Kader, 2005). Its highlighted the seedling vigour and calculated by using the given formula;

$$\text{Germination Index (GI)} = \sum_{i=0}^{k} \frac{ni}{ti}$$

The other seedling observations which included shoot (SL) and root length (RL), seedling length (SDL), seedling dry weight (SDW) and vigour index I (VI I) and II (VI II) were also recorded at 25 days after sowing. Vigour index I and II was calculated as per formula given by Thounaojam and Dhaduk, 2020.

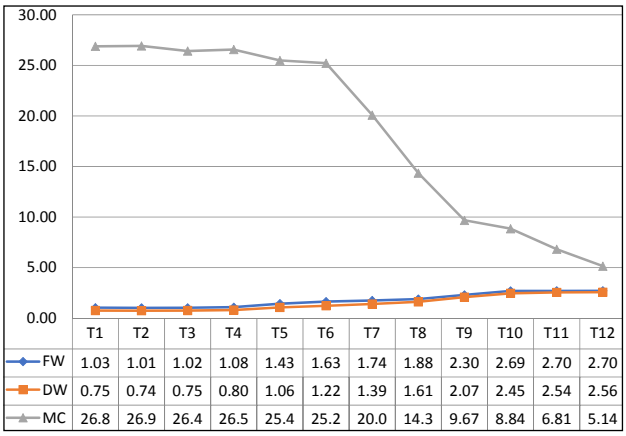
Vigour index-I = Germination percetnage × Seedling length

Vigour index-II = Germination percetnage × Seedling dry weight

The standard statistical analysis was done for analysis of variance as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The results of fresh and dry seed weight with moisture content is shown in Fig. 1. The results showed that fresh weight of ashwagandha seed was gradually increased with an increased in harvesting time up to 10th April (T10, 2.69 g) followed by no more further increased in weight (2.70 g). In case of dry weight continuously increased from the first harvesting (0.75 g) to last harvesting period (2.56 g). The average mean value of fresh and dry 1000 seed weight was1.77 and 1.50 g respectively. There was not much different in moisture content of seed harvested in between the 10th January (T1) to 28th February (T6) with the mean value of 26.25%; later on moisture content was sharply decreased and at last harvesting period (T12) the moisture content was 5.14% and it was 5.22 per cent less as compared to first harvesting time (26.89%).



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
FW	1.03	1.01	1.02	1.08	1.43	1.63	1.74	1.88	2.30	2.69	2.70	2.70
DW	0.75	0.74	0.75	0.80	1.06	1.22	1.39	1.61	2.07	2.45	2.54	2.56
MC	26.8	26.9	26.4	26.5	25.4	25.2	20.0	14.3	9.67	8.84	6.81	5.14

Fig.1: Variability of fresh and dry weight with moisture content in ashwagandha seed harvested at different time period

The impact of moisture content with respect to harvesting period was revealed earlier in various crop like okra (Begum and Ayub. 2022), sunflower (Vladimir *et al*. 2012), oilseed rape (Sadeghi *et al*. 2010), peas (Siddique and Wright, 2003) and soybean (Isaac *et al*. 2016). It is very much important to collect the seed at harvest maturity as it's defines as the moisture declines to a harvestable level in the crops harvested as seed in dry form (TeKrnony and Egli, 1997). The decrease in moisture content in present study is might be due to the increase in dry matter accumulation as advancement of drying maturity (El Balla *et al*. 2011). The similar finding and results were also reported in okra seed harvested at different time (Begum and Ayub, 2022). As a result, 1000 dry seed weight was gradually increased in present investigation. With the progression of physiological maturity, moisture content decrease but increased in dry matter accumulation are being well established phenomenon during seed maturity (Barnwal *et al*. 2017).

The effect of different harvesting period on germination percentage of ashwagandha seed was found significant and its graphical data is shown

in Fig. 2. The mean germination percentage was found 52.11% during the course of present different harvesting period. The seed harvested during the month of January (T1 to T3) showed average germination percentage of 47.63% which was lowest germination rate as compared to February (50.13%, T4 to T6), March (55.49%, T7 to T9) and April month (55.18%, T10 to 12). The significantly maximum germination was found in T7 (56.48%) followed by T10 (56.05%). However, seed harvested from 28th February (T6) till 30th April was recorded significantly at par in terms of their germination percentage. Earlier researchers showed that only 7% seed germination rate in freshly harvested ashwagandha seeds, 40% in scarified seeds and 90% in one year stored seeds mixed with soil and cocopeat (Trivedi and Anupama, 2010). It indicated that ashwagandha seed germination rate are greatly influenced by its physiochemical nature of seed.

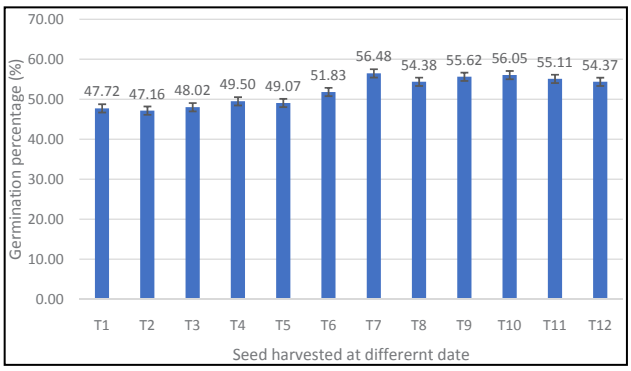


Fig. 2: Germination percentage of ashwagandha seed var. GAA-1

However, in present studies showed that it could increase the germination percentage in freshly harvested seed of more than 50% by timely harvesting the seed. The right choosing of optimal harvesting stage has a great significant effect on seed quality (Begum and Ayub, 2022). The maximum seed quality was attained at physiological maturity after which deterioration started resulting seed germination and vigour declined (Harrington, 1972). However, it was also confirmed that maximum seed quality does not meet exactly at physiological maturity it needs some more time after attaining this stage of maturity to improve the seed quality (Zanakis *et al*. 1994). Seed harvested during the early stage of maturity content more moisture and quickly deteriorate; loo late also becomes over dry. Therefore identification of an ideal harvesting time is so important to have maximum seed quality. Seed size, shape, 1000 seed weight with moisture content are prominently influenced by harvesting stages and these seed morphological traits may be linked with seed germination (Jacobsen *et al*. 2016; Sintim*et al*. 2016). In the same line, the present investigation showed that higher germination percentage in a specific period of seed harvesting even though all seeds showed physiological maturity.

The statistical mean value of other seed quality parameters recorded during the seed germination is shown in Table 2 and their mean squares values are shown in Table 3. It was disclosed that significant different in all observed parameters except shoot length (SL), seedling length (SDL) and shoot dry weight (SDW). Root growth was found maximum in those seed harvested after February. Significantly maximum root length (0.73 cm) was recorded in seed harvested at 10th March (T7) as well as at 20th April (T11). Mean days to germination completion (DGC) in those seed which were harvested during the month of March to April were 18.33 days which was significantly less as compared to January to February with the mean days to germination completion value of 19.83 days. Germination energy is directly influenced with the germination percentage and days to germination completion, seed harvested from March (T7) to April (T12) had more than 3 germination energy value. Moreover significantly maximum germination energy was found in T10 (3.24). However, significantly maximum germination index (GI) was noticed in seed harvested at 10th March (T7: 0.90) and lowest was found in T1 and T5 (0.70). VI I was established continuously increasing from the first harvesting period, 10th January (T1: 146.51) to 10th March (T7: 185.88) after that there was not maintained linearity. However the value of VI I found in seed harvested beyond the 10th March was higher than the seed harvested in month of January and February. Meanwhile, significantly maximum VI I was registered in T7 (185.88) followed by T10 (178.29). In case of VI II, similarly, considerably maximum value was occurred in T7 and T10 (18.35).

The seed embryo is the site of reserve food materials accumulation which includes mainly carbohydrates, lipids and proteins (Yang *et al*. 2022). During the germination time the reserve food materials are hydrolozed and transport with the help of water

Table 2: Effect of seed harvested at different time period on seedling quality parameters of ashwagandha var. GAA-1

Date of harvesting	SL (cm)	RL (cm)	SDL (cm)	DGC (days)	GE	GI	SDW (mg)	VI I	VI II
T1	2.42	0.64	3.06	20.67	2.32	0.70	0.32	146.51	15.10
T2	2.45	0.66	3.10	20.33	2.32	0.78	0.31	146.46	14.76
T3	2.45	0.69	3.19	21.33	2.26	0.75	0.31	153.12	14.94
T4	2.46	0.66	3.12	19.00	2.61	0.76	0.32	154.40	15.90
T5	2.52	0.63	3.15	19.00	2.59	0.70	0.32	154.58	15.80
T6	2.51	0.69	3.20	18.67	2.83	0.88	0.32	165.74	16.78
T7	2.55	0.73	3.29	18.33	3.08	0.90	0.32	185.88	18.35
T8	2.55	0.71	3.16	18.00	3.02	0.88	0.32	171.72	17.54
T9	2.48	0.72	3.20	18.67	2.99	0.88	0.31	177.83	17.38
T10	2.51	0.72	3.18	18.33	3.24	0.78	0.33	178.29	18.35
T11	2.49	0.73	3.17	18.17	3.22	0.79	0.32	175.00	17.80
T12	2.51	0.72	3.13	18.50	3.17	0.79	0.33	170.02	17.92
S.Em±	0.06	0.02	0.09	0.57	0.11	0.03	0.01	6.90	0.60
C.D. (5 %)	NS	0.06	NS	1.66	0.33	0.09	NS	20.15	1.76
CV	4.13	5.01	4.67	5.17	7.03	6.60	3.71	7.25	6.24

Table 3: Mean squares of various germination related parameters of ashwagandha seed harvested at different time period

Source of variation	df	GP	SL	RL	SDL	DGC	GE	SDW	VI I	VI II
Treatment	11	125.81	0.005	0.006	0.010	5.70	0.53	0.0001	1501.47	14.42
Error	24	5.06	0.011	0.002	0.022	0.75	0.02	0.0001	103.89	0.72

#df = Degree of freedom; Treatment: Seed harvested at different date.

Fig. 3: (A) Ashwagandha plant **(B)** Harvested matured berries enclosed by papery calyx **(C)** Matured berries **(D)** Extracted seed from matured berries **(E)** Germinated seedling

(Kaur *et al*. 2021). Those seeds harvested in the month of March to April showed higher dry weight which indicated that more accumulation of reserve foods correspondingly higher shoot length, root length and seedling length in present study. In the same line, with differed in seed dry weight at different harvesting periods significantly increased the seedling length in sunflower (Mahesha *et al*. 2001), lentil (Khatun *et al*. 2009), groundnut (Gaikwad, 2014), *Seriphidium transiliense* (Bademuqiqige *et al*. 2018) cucumber (Gupta *et al*. 2021) and maize (Aswin *et al*. 2023) during the seedling stage.

The higher germination index means seeds germinate at faster rate complete the germination process in less duration. This might be due to the results of better dry matter accumulation with an optimum moisture level in such seeds harvested at this maturity period. Results showed that higher seed vigour in terms of seedling length and dry weight in various crop during the seedling stage (Aswin *et al*. 2023; Gupta *et al*. 2021; Khatun *et al*. 2009).

CONCLUSION

From the present investigation it can be concluded that ashwagandha seed harvested at different time period had significant impact on seed germination and its seed quality. To get maximum seed germination rate of freshly harvested seed naturally it could be harvest during the month of March.

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