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Physiological and biochemical changes during seed development and maturation in onion *(Allium cepa L.)*

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Abstract : Onion (*Allium cepa* L.) is one of the important commercial bulb vegetables. Harvesting a seed crop at the correct stage not only aims at preserving the viability and vigour potential of the seeds but also eliminates the field damage. The present study was made to know physiological and biochemical changes associated during seed development and the right stage of harvest for quality seeds. The samples were collected from the tagged umbles starting from 5th day after 50% flowering up to 45th day. Dry weight of the seeds increased and reached the maximum (0.31g) at 45 DAA (days after anthesis). Dehydrogenase and alpha amylase activity increased progressively with accumulation of seed reserves. Peroxidase activity was almost unaltered from 20 to 40 DAA and later decreased at 45 DAA. SDS-PAGE profile of Tris soluble storage proteins showed synthesis and accumulation of several polypeptides during the course of seed maturation. The seed quality in terms of germination and vigour were similar and their maximum at 40 and 45th DAA. The seed attained physiological maturity between these stages and 45th DAA with seed moisture content of 16.6% was found to be the right stage for harvest.

Key words: Onion, Enzyme activity, Physiological maturity, Germination

Introduction

Onion (*Allium cepa* L.) is one of the important commercial bulb vegetables grown in India. In any crop, the stage of harvest greatly influences the quality of the seeds produced. For seed purpose, physiological maturity is the right stage (Harrington 1972) and the maturity stages have been found to induce several physiological and biochemical changes during seed growth and development (Hoover and Dennison 1953). These changes play a major role in determining viability and vigour potential of seed. Thus, seed is a suitable material to study the function of space and stage specific proteins (Goldberg *et al*. 1989). Hence, a study was carried out in this direction to find out various physiological and biochemical changes that occurred during seed development and to identify right stage of harvest for quality seeds in onion *cv*. Arka Bindu.

Materials and Methods

Onion *cv*. Arka Bindu was grown in the *Kharif* season for production of bulbs and selected bulbs were planted in *rabi* 2004-05 for the experimental purpose. The crop was raised using recommended package of practices. For seed developmental studies, individual umbels were tagged at 50% anthesis during the flowering period. The samples were collected from the tagged umbels, at 5 days interval, starting from 5th day after 50% flowering up to 45th day. Various physiological and biochemical studies were conducted as per the procedures given below.

The moisture content of seeds was determined using halogen moisture analyzer calibrated to oven drying. For 100 seed fresh weight, seeds collected at different stages were weighed immediately. These seeds were dried using halogen moisture analyzer and dry weight was recorded. Seed yield of 5 randomly selected plants at various stages of seed development was recorded in grams and average seed yield per plant was worked out. The seeds were evaluated for germinability (ISTA 1996) and vigour index (Abdul Baki and Anderson 1973). The 100-seed weight was determined following the ISTA rules (ISTA 1996). The seed steeped water at 100° C was used for estimation of EC (Agrawal and Dadlani 1987). Total starch content, Amylase activity, Peroxidase activity (Sadasivam and

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Manickam 1996), Protein content (Lowry *et al*. 1951) and Dehydrogenase activity (Agrawal and Dadlani 1987) were studied during various developmental stages. For SDS-PAGE exactly 0.1g of sample was homogenized in an eppendarf tube by adding 400ml of extraction buffer (Tris–glycine pH 8.3). The samples were centrifuged at 10000× g for 10 min. and supernatant was collected. To 10 ml of the supernatant, 10ml of working sample buffer was added and boiled at 100°C for 5 minutes. The samples were subjected to SDS-PAGE (Laemmli 1970) on 12.5% acryl amide gels.

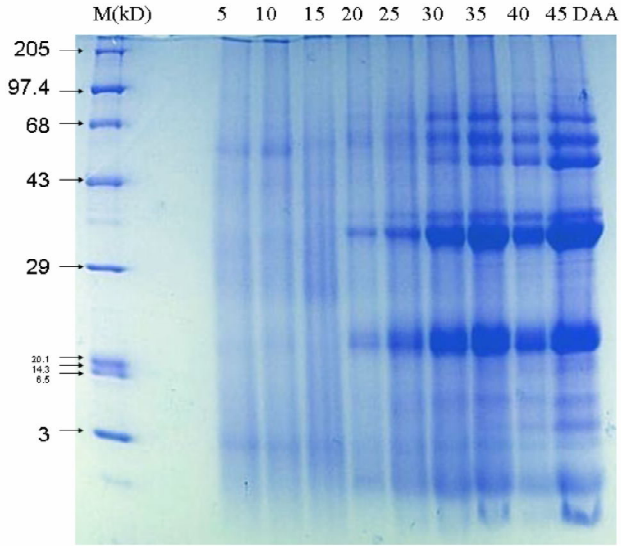
The data were statistically analysed as per the methods outlined by Sundararaj *et al*. (1972) adapting "Fisher's Analysis by Variance Technique. Critical Difference (CD) values were computed wherever 'F' test was significant.

Results and Discussion

During seed development and maturation, many events like formation of protein, starch and changes in enzymes activities were noticed (Table 1). Protein accumulation occurred till 35 DAA (57.4mg/ml), thereafter it remained almost constant. Starch accumulation increased from 20 DAA (33.8mg/ml) to 45 DAA (86.4mg/ml) imparting more weight to the seeds with advancement in maturation. The process of reserve material accumulation during maturation was further accompanied by loss in seed moisture content (Table 2).

Appearance of enzyme activity varied with stage of maturation. Dehydrogenase and α-amylase activity increased progressively with accumulation of seed reserves (Table 1). The dehydrogenase activity was noticed only at 20 DAA (0.23) and increased significantly and progressively upto 45 DAA (1.81). The alpha amylase activity was very less during initial stages of seed development (10 and 15 DAA) and increased from 20 DAA (460mg maltose produced /ml/min.) to 40 DAA (1780mg maltose produced /ml/min.). Peroxidase activity as almost unaltered from 20 DAA (0.9) to 30 DAA (0.8) was next at 35 DAA and later decreased upto 45 DAA (0.5). The decrease in whole seed peroxidase activity (45 DAA) when compared to 35 DAA could be attributed to loss in seed moisture or due to alteration in organ specific peroxidase activity as noticed by Rauf (1980a and 1980b).

SDS-PAGE profile (Fig. 1) of Tris soluble storage proteins showed synthesis and accumulation of several polypeptides. Rapid accumulation of polypeptides was noticed from 20 DAA with synthesis of new



M - Marker (Myosin-205, Phosphorylase B-97.4, BSA-68, Ovalbumin-43, Carbonic anhydrase-29, soybean Trypsin inhibitor-20.1, Lysozyme-14.3, A-protinin-6.5, Insulin-3 kD.)

Fig 1: Developmental changes in the accumulation of seed protein in onion Cv. Arka Bindu seperated on 12.5% SDS-PAGE

Table 1. Biochemical changes during seed development of onion cv. Arka Bindu

Treatment	Protein (µg/ml)	Starch (µg/ml)	Dehydrogenase (O.D 510nm)	Peroxidase (△OD/min /mg protein)	Amylase (µg maltose prod. ml/min)
10 DAA	35.1	30.4	0	0	60.0
15 DAA	37.9	35.8	0	0	140.0
20 DAA	41.0	33.8	0.2	0.9	400.0
25 DAA	43.9	38.1	0.3	0.9	1100.0
30 DAA	52.5	39.0	0.4	0.8	1060.0
35 DAA	57.4	42.9	0.8	1.1	1480.0
40 DAA	57.6	75.3	1.1	0.7	1780.0
45 DAA	56.2	86.4	1.8	0.5	1480.0
SEm ±	1.4	1.9	0.01	0.2	92.2
CD at 5%	2.8	4.0	0.03	0.3	190.3
CD at 1%	3.8	5.5	0.03	0.4	257.9

DAA = Days after anthesis

Table 2. Changes in seed fresh weight, dry weight, test weight, initial moisture content and per plant yield as influenced by seed development stages in onion cv. Arka Bindu

Treatments	100 seeds Fresh weight (g)	100 seeds Dry weight (g)	100 seeds weight (g)	Initial moisture content of seed (%)	Seed yield per plant (g)
15 DAA	-	-	-	-	-
20 DAA	0.73	0.15	0.16	79.03	2.18
25 DAA	0.72	0.18	0.19	78.90	2.39
30 DAA	0.76	0.17	0.18	78.33	2.82
35 DAA	0.81	0.24	0.25	70.45	4.01
40 DAA	0.57	0.27	0.29	52.88	4.84
45 DAA	0.37	0.31	0.33	16.60	5.57
SEm ±	0.02	0.02	0.02	2.49	0.34
CD at 5%	0.04	0.03	0.03	5.19	0.69
CD at 1%	0.06	0.05	0.05	7.06	0.95

DAA = Days after anthesis

Table 3. Seed quality as influenced by seed developmental stages in onion cv. Arka Bindu

Treatments	Germi-nation (%)*	First count (%)*	Seedling Root length (cm)	Seedling Shoot length (cm)	Seedling Vigour Index	EC (µs/cm)
15 DAA	0.0 (0.0)	0.0 (0.0)	0	0	0	157.7
20 DAA	1.0 (2.9)	0.0 (0.0)	0	0	0	152.7
25 DAA	7.0 (14.9)	2.5 (8.9)	0	0	0	157.6
30 DAA	10.5 (18.8)	4.0 (11.3)	0	0	0	102.8
35 DAA	45.5 (42.3)	14.5 (22.3)	5.2	6.2	518	134.5
40 DAA	95.0 (76.9)	77.0 (61.2)	6.4	8.9	1456	128.6
45 DAA	96.0 (78.4)	83.0 (65.6)	6.7	8.9	1474	97.3
SEm ±	2.5	1.3	0.3	0.3	50	12.0
CD at 5%	5.25	2.73	0.6	0.6	104	25.8
CD at 1%	7.15	3.72	0.8	0.9	141	35.8

* Figures in the paranthesis are arc sine transformed values.

polypeptides till 40 DAA and remained unchanged at later developmental stages. Two polypeptides ranging between 43-29 kD and 29-21 kD stained intensively with CBB suggesting their abundant accumulation during seed maturation. A total of sixteen polypeptides appeared at 40 and 45 DAA and they remain unchanged till maturation.

The changes in dry weight, test weight and initial moisture content are shown in Table 2. The 100 seed weight increased from 20 DAA (0.16g) to 45DAA(0.33g) and the dry weight of seeds increased gradually from 20 DAA (0.15g) to 40DAA(0.27g). The moisture content was reduced drastically from 52.9% to 16% at 45th DAA. The 100 seed weight and dry weight of seeds increased as the stage of harvest advanced and reached the maximum at 40 DAA after which the values remained almost constant. At this stage dry matter accumulation mainly in the form of starch and protein was also at its maximum (Table 1). The dry weight of seeds remained almost constant after 40DAA indicating that the seeds attained physiological maturity at 40 and 45 DAA. The moisture content was reduced drastically from 52.9% at 40th DAA to 16% at 45th DAA. Similar findings in onion were also reported by Steiner and Akintobi (1986) The seed yield per plant was maximum (5.57 g) at 45th DAA.

The seed quality parameters in terms of germination and vigour were given in Table 3. All the quality parameters improved with increased in seed maturity from 15 DAA to 40 DAA and remained almost constant after that. Germination was observed only at 25 DAA (7%) and rapid increase in germination was noticed after 30 DAA and reached maximum at 40 DAA (95%) and it remained almost constant at 45 DAA (96%). Since there was no or few seeds germinated at early stages of seed development the vigour index was not recorded till 30 DAA. Among the remaining later stages of seed development, maximum vigour index was recorded at 45 DAA (1474). The electrical conductivity of seed leachates significantly decreased as the stage of seed development advanced.

The continued development of immature seeds during the course of seed development as reflected in accumulation of food reserves and increase in test weight is the reason for better germinability with seedling vigour as measured in terms of seedling root and shoot length. Similar observation was recorded by Thomazelli *et al*. 1994, Shantha and Pandita 2002 in onion. Hence, it was concluded that 45 DAA was the ideal stage of harvest for quality seeds and onion umbels in *cv*. Arka Bindu can be harvested at 126 days after bulb planting for maximum seed quantity and quality. Similar to our observation Kalavati *et al*. (1990) also reported that the seed crop of Bellary onion could be harvested at 125 days after bulb planting for obtaining high quantity and quality seeds.

In summary, the onion seed *cv*. Arka Bindu attained physiological maturity between 40 and 45 DAA and 45th DAA with seed moisture content of 16.6% was found to be the right stage for harvest.

सारांश

प्याज एक महत्वपूर्ण व्यावसायिक बल्ब सब्जियों में से है। वर्तमान अध्ययन के लिए फसल को सही मंच के विकास के दौरान शारीरिक

और जैव रासायनिक परिवर्तन बीज और गुणवत्ता के बीज के लिए बनाया गया था। नमूने टैग 50% करने के लिए 45 दिन बाद फूल 5 दिन से शुरू अम्बेल्स से एकत्र किए गए थे। बीज की सूखी वजन में वृद्धि हुई थी और 45 में अधिकतम (0.31 ग्राम) डीडीए पर पहुंच गया। डिहाइड्रोजनेज और अल्फा एमाइलेज गतिविधि बीज भंडार के संचय के साथ उत्तरोत्तर वृद्धि हुई है। परआक्सीडेज गतिविधि लगभग 20 से 40 डीडीए से अनछुए था और बाद में 45 डीडीए कम। ट्रिस घुलनशील प्रोटीन के एसडीएस प्रोफ़ाइल से पता चला कि बीज परिपक्वता के समय कई पालिपेक्टाइड का संश्लेषण होता है। बीज अंकुरण और शक्ति के मामले में 40 और 45 डीडीए गुणवत्ता में समान हैं। इन चरणों के बीच बीज की शारीरिक परिपक्वता हासिल होती है। और 45 डीडीए पर मोस्चर कन्टेन्ट 16.6% पाया गया।

REFERENCES

Abdul Baki AA, Anderson JD (1973) Vigour determination in soybean seed by multiple criteria. Crop Sci 13: 630-633.

Agrawal PK, Dadlani M (1987) Techniques in Seed Science and Technology, South Asian Publishers, New Delhi.

Goldberg RB, Baker SJ, Perez-Grau L (1989) Regulation of gene expression during plant embryogenesis. Cell, 56: 149-160.

Harrington JF (1972) Seed storage and longevity. In: Kozlowski TT (Ed) Seed Biology, vol III, Academic press, New York, pp 145-245.

Hoover MW, Dennison RA (1953) The correlation of stages of maturity with certain physiological measurements in southern pea (*Vigna sinensis*). Proc Amer Soc Hort Sci 62: 391-396.

ISTA (1996) International Rules for seed testing. Seed Sci Technol (Supplement) 24: 1-255.

Kalavati D, Vanangamudi K, Daramoorthy K (1990) Influence of date of harvest and method of bulb planting on the seed quality in Bellary onion. Madras Agric J 77: 61-63.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.

Lowry H, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-75.

Rauf A (1980 a) Comparative growth and biochemical studies on seed development. 5. Protease and a-amylase. Pl Biochem J 7(2): 167-172.

Rauf A (1980 b) Comparative growth and biochemical studies on seed development. 6. Peroxidase and IAA-oxidase. Pl Biochem J 7(2): 173-177.

Sadasivam S, Manickam (1996) Biochemical methods. New Age International Publication Private limited, New Delhi.

Shantha Nagarajan, Pandita VK (2002) Development of desiccation tolerance and quality during seed maturation in fresh and shade dried seeds of onion. Seed Tech News 32(1): 18-20.

Steiner JJ, Akintobi DC (1986) Effect of harvest maturity on viability of onion seed. Hort Sci 21: 1220-1221.

Sundararaj N, Nagaraju S, Venkataraman MN, Jagannath MK (1972) Design and analysis of field experiments, UAS, Bangalore.

Thomazelli LF, SILVA RFD, Sediyamma CSB, Foaro ID (1994) Onion seeds: harvesting at the right time. Agropecuaria catalinenise 4: 22-23.