Seed Research 46(2): 147-151, December, 2018

Physiological and Biochemical Changes during Seed Maturation of Bottle gourd

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(Received : July 2018; Revised : October 2018; Accepted : October 2018)

ABSTRACT: The poor seed quality of bottle gourd seed is due to due to non-synchronous fruit setting in bottle gourd and all the fruits do not reach maturity at the time of plant senescence resulting in mixture of mature and immature seeds. This results in poor seed yield and reduction in seed quality. The present investigation was conducted for two years during 2014 and 2015 at Punjab Agricultural University, Ludhiana to determine the physiological maturity of bottle gourd (*Lagenaria siceraria*) seeds in relation to fruit age and pre-storage duration. The fruits were harvested at 25, 35 and 45 days after pollination (DAP) and stored for 0, 10, 20 and 30 days before extraction of seed. Each female flower was tagged date wise after doing pollination with hand to monitor fruit age. Numbers of developed seeds, 100 seed weight, germination, speed of germination, seedling length and vigour indices, total lipids of seeds increased with delay in harvesting and increase in the pre-storage duration in both the years. Maximum seed weight, germination, vigour indices, lipid content were observed when fruits were harvested 45 DAP and pre-stored for at least 10 days in both the years. However, sugar content of the seeds decreased with delay in fruit harvesting and pre-storage duration.

Keywords: Bottle gourd, Seed quality, Pollination, Vigour, Germination, Physiological maturity

Bottle gourd [(*Lagenaria siceraria* (Molina) Standl.] is an important cucurbitaceous crop grown for its fleshy fruits in tropical and subtropical regions which is consumed either raw (e.g. *Cucumis melo)* or cooked. In some parts of the world, cucurbit seeds are produced and consumed as snack food because of their high nutritive values. However, most of seed production is directed to plant propagation. However, seed production in this crop is more complicated than in dry-seeded grain crops, because seed matures within the moist fruit and are often held at high moisture content for several weeks before harvest [1]. Farmers generally harvest the fruits of this species after complete senescence of the plants, but due to its monoecious flower nature, the female flowers appearance difference is more. Therefore, all the fruits do not reach maturity at the same stage before plant senescence, resulting in a mixing of mature and immature seeds at the time of harvest [2]. This results in poor seed yield and low germination rate [3]. The yield of viable and vigorous seed is the ultimate goal for seed producer [4].

In general, the three major phases during seed development are; 1. rapid gain in fresh weight because of cell division and early expansion; 2. rapid dry weight accumulation driven by cell enlargement; 3. loss of fresh weight during drying. Seed development in fleshy fruits (e.g. cucurbits) differs primarily because phase II of seed development is extended over a much longer time compared to dry seeded grain crop [1]. Cucurbits seed maturity in fruits occurs over many days after pollination and depends primarily on temperature during development and does not require desiccation in order to mature and transition from developmental to germinative mode [5]. The stage of maturity determines storage potential of seeds. Early harvested seeds are immature and poorly developed and are poor storer compared to seed harvested at proper physiological maturity [6, 7]. It is also argued that fruits harvested before obtaining physiological maturity and allowed for ripening of seed in the fruit for few days also produce good quality seed [2]. The pre-storage duration is an important aspect of improving the seed quality by retaining seeds in the fruit after its detachment from the plant. Since, the development of seed continues in the fleshy fruit due to continuous supply of nutrients from fruit to seeds, there is need to study various physiological and biochemical changes associated with various fruit age and pre-storage duration. Thus, keeping in view of the justification explained above, the present

investigation was conducted to ascertain the effect of stage of fruit harvest and pre-storage duration on optimum seed viability and vigour in bottle gourd seed.

MATERIALS AND METHODS

The seed crop of bottle gourd variety Punjab Komal was raised at the Department of Vegetable Sciences, P.A.U. Ludhiana in 2014 and 2015. Seed was sown in polythene bags in second week of February and transplanted in second week of March in 2014 and 2015, respectively. Experiment was conducted in factorial randomized block design. The first factor was three harvest stages (25, 35 and 45 days after pollination) and second factor was storage duration (0, 10, 20 and 30 days after harvest). Each female flower was date-tagged at anthesis to monitor fruit age and fruit harvested at 25, 35 and 45 days after pollination (DAP) by insect based on pollination at the day of flower opening and then stored for 0, 10, 20 and 30 days at room temperature, before extracting the seeds. Two fruits per vine were retained. After that the seeds were extracted from these fruits, washed, dried under shade-net and stored. Five fruits per replication in each treatment were selected for recording observation. Observations were recorded on total number of seeds, seed weight per fruit (g), percentage of developed seeds (Filled seeds were considered developed seeds and unfilled seeds as undeveloped seeds) and 100 seed weight (g). Germination was conducted as per ISTA rules [8]. Various physiological parameters *viz*; speed of germination ([9], seedling length (cm), seedling dry weight (g) and seedling vigour index [10] were recorded. Speed of germination was calculated as: S (Gt/Tt) where

Gt = number of seeds germinated or emerged on t^{th} day

Tt = number of days up to t^{th} day

Seedling vigour was calculated as: (percent germination x total seedling dry weight). Biochemical parameters *viz.*, total soluble sugars [11], total lipids [12] and relative water content (%) [13] were estimated from the seed extracted at each stage of harvest. The analysis of variance of factorial randomized block design was analysed [14]

RESULTS AND DISCUSSION

Effect of Stage of Fruit Harvest on Seed yield, Physiological and Biochemical Parameters of Bottle gourd

The data for two years had the same trend for all the characters, therefore, pooled data for two years are given in the tables. The stages of fruit harvest had significant

Table 1. Effect of fruit age and pre-storage duration on seed yield parameters of bottle gourd

S. No.	Treatments	Number of seeds/ fruit	% age of developed seeds	100 seed weight (g)	Seed weight per fruit (g)
Stage of harvest					
1.	H_1 – 25 days after pollination	200.10	42.20	8.285	12.59
2.	H_2 – 35 days after pollination	201.00	77.53	10.23	18.27
3.	H_3 – 45 days after pollination	211.91	81.28	10.75	20.79
	CD (p=0.05)	NS	2.95	0.70	2.03
Pre-storage durations					
1.	S_0 – Immediate extraction	199.00	47.16	6.56	12.11
2.	S_1 – after 10 days	204.66	60.13	8.93	16.78
3.	S_2 – after 20 days	213.83	75.50	9.53	19.47
4.	S_3 – after 30 days	199.83	85.23	10.72	20.51
	CD (p=0.05)	NS	3.35	0.81	2.25
Interactions					
1.	$H_1 \times S_0$	191.00	12.60	4.36	5.99
2.	$H_1 \times S_1$	196.83	27.53	6.17	10.07
3.	$H_1 \times S_2$	218.83	50.91	8.38	16.74
4.	$H_1 \times S_3$	193.66	77.74	9.65	17.56
5.	$H_2 \times S_0$	183.83	58.82	7.20	11.64
6.	$H_2 \times S_1$	208.50	77.22	10.47	20.45
7.	$H_2 \times S_2$	215.17	86.15	9.85	20.38
8.	$H_2 \times S_3$	196.50	87.94	10.82	20.60
9.	$H_3 \times S_0$	222.17	70.04	8.12	18.69
10.	$H_3 \times S_1$	208.66	75.65	10.15	19.80
11.	$H_3 \times S_2$	207.50	89.42	10.35	21.28
12.	$H_3 \times S_3$	209.33	90.00	11.70	23.40
	CD (p=0.05)	NS	5.80	1.45	3.35
	CV%	6.35	7.25	4.95	5.40

effect on the development of seed. Total number of seeds were not influenced by the stage of harvest. This may be due to the fact that seed had formed within 15-20 days after pollination and after that only filling of seed occurs. Percentage of developed seeds, seed weight per fruit and 100-seed weight were maximum in the fruit harvested at 45 DAP (Table 1) and significantly better than 35 and 25 DAP. The seed quality traits like germination, speed of germination, seedling length, seedling dry weight and vigour indices (Table 2) were significantly higher when fruits harvested at 45 DAP, while the seed moisture content reduced significantly with delay in harvest and maximum with 25 DAP. The total lipids were increased significantly with delay in harvesting and maximum was observed at 45 DAP, while sugar content was reduced drastically at 45 DAP (Table 3).

Accomplishment of maximum seed quality at the end of seed filling is must to get maximum viability and vigour of seed. The possibility of increasing percentage of developed seeds, seed weight, germination, vigour indices and lipids with increasing fruit age might be due to completion of physiological maturity of seeds. The progressive increase in size of seed due to delay in harvesting results in accumulation of nutrients from the mother plant to the seeds. The reduction of sugar content in mature seed is mainly due to its conversion in starch. Similar findings were reported in bottle gourd [15] and in watermelon [16].

Effect of Pre-storage Duration on Seed yield, Physiological and Biochemical Parameters of Bottle gourd

The maturity of bottle gourd seeds continue in the fruit even after its detachment from the mother plant. Data presented in tables 1-4 revealed that increase in pre-storage duration resulted increase in seed quality. Hundred seed weight, percentage of developed seeds,

Table 2. Effect of fruit age and pre-storage duration on seed germination and vigour of bottle gourd

S. No.	Treatments	Germination (%)	Speed of germination	Seedling length (cm)	Seedling dry weight (g)
Stage of harvest					
1.	H_1 – 25 days after pollination	20.42	0.71	4.95	0.09
2.	H_2 – 35 days after pollination	53.96	2.57	15.58	0.28
3.	H_3 – 45 days after pollination	72.09	3.44	36.33	0.55
	CD (p=0.05)	4.35	0.33	2.05	0.029
Pre-storage durations					
1.	S_0 – Immediate extraction	15.00	0.65	9.46	0.20
2.	S_1 – after 10 days	41.95	1.70	13.43	0.25
3.	S_2 – after 20 days	61.12	2.85	18.87	0.35
4.	S_3 – after 30 days	77.22	3.76	22.09	0.38
	CD (p=0.05)	5.50	0.36	2.45	0.036
Interactions					
1.	$H_1 \times S_0$	0.00	0.00	0.00	0.00
2.	$H_1 \times S_1$	0.00	0.00	0.00	0.00
3.	$H_1 \times S_2$	30.00	4.60	4.60	0.16
4.	$H_1 \times S_3$	51.67	7.32	7.32	0.20
5.	$H_2 \times S_0$	13.33	3.68	3.68	0.14
6.	$H_2 \times S_1$	49.17	11.54	11.54	0.24
7.	$H_2 \times S_2$	70.00	16.07	16.07	0.33
8.	$H_2 \times S_3$	83.33	19.68	19.68	0.40
9.	$H_3 \times S_0$	31.67	24.70	24.70	0.45
10.	$H_3 \times S_1$	76.67	28.76	28.76	0.52
11.	$H_3 \times S_2$	83.34	35.94	35.94	0.57
12.	$H_3 \times S_3$	96.67	39.28	39.28	0.63
	CD (p=0.05)	9.12	4.02	3.65	0.05
	CV%	3.05	2.55	3.07	2.37

seed weight per fruit, germination, speed of germination, seedling length, seedling dry weight, vigour indices and total lipids were maximum when pre-stored for 30 days. However, total sugar and seed water content were reduced at this stage which is desirable from seed quality point of view. This data clearly indicated that seeds continue to mature within the fruit even after its detachment from the mother plant. The enhanced pre-storage period provides an opportunity to the immature seed to draw nutrients from the fruit flesh and become viable and mature seed further improves its seed quality [5]. The increased seed weight with increase in storage period also helps to increase the vigour. The fleshy fruits protect and nurture seeds until they are fully mature. In cucurbits, there is a progressive increase in accumulation of assimilates during fruit/ seed maturation [16]. In *Cucumis sativus*, a post harvest storage of 10-15 days yield resulted in better quality seed [17].

Interaction Effect of Stage of Fruit Harvest and Pre-storage Duration

The interaction effect of stage of harvest and pre-storage duration (H x S) were also found to be significant for all the characters. The number of seeds per fruit were non-significant. Percentage of developed seeds, 100 seed weight and seed weight per fruit were maximum with H_3S_3 and significantly at par with H_3S_2 (Table 1). Physiological parameters like germination, speed of germination, seedling length, seedling dry weight, vigour index-I were also maximum when fruits were harvested at 45 DAP and pre-stored for 30 days and minimum with H_1S_0 (Table 2). It was also observed that seeds obtained after 35 DAP and pre-stored for 30 days resulted in 83.33% germination but the vigour of this seed was significantly low. The seed reserve i.e. total lipids, starch were significantly better at H_3S_3, while total sugar was minimum at this stage. Seed relative water content was also reduced significantly at H_3S_3 indicating maturity of

Table 3. Effect of fruit age and pre-storage duration on vigour and biochemical traits of bottle gourd seed

S. No.	Treatments	Seedling dry weight (g)	Vigour index I	Relative water content (%)	Total soluble sugars (mg/g dry wt)	Total lipids (%)
Stage of harvest						
1.	H_1 – 25 days after pollination	0.09	131.81	7.92	13.75	0.91
2.	H_2 – 35 days after pollination	0.28	853.63	7.3	9.08	2.08
3.	H_3 – 45 days after pollination	0.55	2445.5	6.86	6.95	4.05
	CD (p=0.05)	0.03	157.62	0.35	141	0.28
Pre-storage durations						
1.	S_0 – Immediate extraction	0.20	276.45	8.99	15.00	1.61
2.	S_1 – after 10 days	0.25	926.24	8.01	11	2.21
3.	S_2 – after 20 days	0.35	1430.8	7.13	7.63	2.55
4.	S_3 – after 30 days	0.38	1941.0	5.32	6.08	3.07
	CD (p=0.05)	0.033	175.80	0.54	0.80	0.39
Interactions						
1.	$H_1 \times S_0$	0.00	0.00	9.65	20.38	0.40
2.	$H_1 \times S_1$	0.00	0.00	8.58	15.27	0.81
3.	$H_1 \times S_2$	0.16	146.38	7.82	10.84	1.11
4.	$H_1 \times S_3$	0.20	380.85	5.64	8.50	1.34
5.	$H_2 \times S_0$	0.14	49.44	8.91	14.35	1.27
6.	$H_2 \times S_1$	0.24	564.89	7.96	10.18	2.09
7.	$H_2 \times S_2$	0.33	1148.9	7.06	6.60	2.32
8.	$H_2 \times S_3$	0.40	1651.1	5.25	5.2	2.87
9.	$H_3 \times S_0$	0.45	779.93	8.40	10.27	3.19
10.	$H_3 \times S_1$	0.52	2213.8	7.48	7.55	3.74
11.	$H_3 \times S_2$	0.57	2997.2	6.49	5.45	4.26
12.	$H_3 \times S_3$	0.63	3791.1	5.08	4.53	5.03
	CD (p=0.05)	0.05	315.24	0.51	3.12	0.80
	CV%	2.38	2.67	2.87	2.51	2.37

seed and its storage for longer period (Table 3). During seed development, water content increases and contributes 80% of the seed. But when seed reaches to maturity, it accumulates seed reserves and diminishes the water content [18]. There was lesser germination percentage in early harvested fruits which may be due to presence of more number of immature seeds in cucurbits. Seeds continued to draw the nutrients from the flesh of the fruit and helped in the accumulation of food reserve that resulted in high speed of germination and vigour in late harvested fruits and increased pre-storage duration [18, 19]. Speed of germination and seedling length were more in bottle gourd seeds that were extracted 50 days after anthesis and pre-stored for 60 days after harvest [20]. Lipid peroxidation has a considerable potential to damage the membrane and may be very important in the deterioration of seeds and consequently reduced seed vigour [21]. There was a decrease in the amount of total soluble sugars which may be due to conversion of sucrose into starch [22].

From the data, it is evident that delay in harvesting and increasing the pre-storage duration resulted in increased germination, vigour and seed reserve material of bottle gourd seed. Therefore, from the present study, it may be concluded that the fruits of bottle gourd (*Lagenaria siceraria L*.) should be harvested at 45 DAP and stored for at least 10 days to get higher seed yield and better seed quality.

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