THE SIZE AND GERMINATION OF EGGPLANT SEED IN RELATION TO FRUIT MATURITY AT HARVEST, AFTER-RIPENING AND ETHYLENE APPLICATION

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Abstract. In eggplant cultivars Black Beauty, Emi, Long Negro and Tsakoniki cultivated for seed, flower induction and flower weight decreased in the presence of developing fruit on the plant. Harvesting prior to maturity (25-35 days after anthesis), aimed at increasing flower induction and fruit set, resulted in small seeds that failed to germinate or germinated poorly. When these fruit were stored for 20 days at 25±3^{0}C before seed extraction, seed size and germination increased indicating seed filling and maturation ('after-ripening') within the harvested fruit. In year 1, a single application of ethylene before storage increased the germination of Black Beauty and Long Negro harvested 25-35 days after anthesis, but reduced that of Emi and Tsakoniki. In year 2, ethylene application once before the storage of fruits harvested 35 days after anthesis promoted the germination of Tsakoniki and Emi, and when ethylene was applied three times germination was increased further. The possible applications of early harvest, fruit storage prior to seed extraction and ethylene treatment to eggplant seed production are discussed.

Keywords: aubergine, *Solanum melongena* L., non-climacteric fruit

INTRODUCTION

Eggplant (*Solanum melongena* L.), a species indigenous to Southern Asia, is a major vegetable crop of countries bordering the Mediterranean Basin, particularly Egypt, Turkey and Italy, where annual production is estimated to be 1.0, 0.9 and 0.4 million tonnes, respectively [4]. In addition to its use as a fresh vegetable, eggplant is a potentially important source of antioxidants [13]. Although most eggplant cultivars grown in Greece are imported F_1 hybrids, local varieties and landraces are also cultivated. The advantages of using local varieties are that they are adapted to the climate of the region and favored on the market; additionally the use of local seed reduces the dependence on foreign imports and the loss of foreign exchange.

Eggplant exhibits extensive phenotypic diversity, particularly with respect to fruit morphology [2]. Within the Mediterranean Basin, important centres of eggplant diversity include Turkey [6] and Spain [10, 17]. In Greece, Tsakoniki (also marketed as Argitiki) is the most important domestic, field-grown variety and is considered to be of potential value to eggplant breeders for hybrid development [2, 9]. Other local Greek varieties include Emi, which is a black flask-type eggplant for cultivation under cover.

For seed production of eggplant (*Solanum melongena* L.) fruit are normally harvested at full maturity, which is about 50-60 days after anthesis depending on the cultivar, climate and growing conditions [3, 5, 18, 21]. Seeds extracted from immature fruit show either a lack or a low level of germination.

During fruit development and maturation, there is competition between fruits on the plant, leading to reduced seed size [12, 16]. It would therefore be an advantage to the seed producer if he/she could harvest at the earliest possible time after anthesis and not retain fruit on the plant beyond that date. Although eggplant is a non-climacteric species and fruits do not ripen after harvest, the seeds within the harvested fruit may continue to develop and mature, leading to improved germination [15]. In the present paper we examine whether ethylene application during this 'after-ripening' process will further improve germination.

MATERIALS AND METHODS

Seeds of eggplant (*Solanum melongena* L.) cv. Emi, Long Negro, Tsakoniki and Black Beauty, produced at the Agricultural University of Athens, were sown on 10 February (year 1) in seed boxes containing a peat-based substrate (Klasmann-Deilmann TS-2) and maintained at 25^{0}C day, ≥15^{0}C night. At the cotyledon stage (20 days after sowing), seedlings were transplanted to 0.1 l pots containing the same substrate. Three months after sowing (7 May) 35 plants per cultivar were transplanted to 11 l pots containing a substrate of peat and sand (2:1 v/v) and placed in random blocks in an unheated greenhouse (33±5.5^{0}C day, 16±4^{0}C night) at a plant density of 25,000 plants/ha. Plants were pruned so as to retain a single stem, which was trained on a vertical cordon. Irrigation and fertilizer application was carried out via a drip system according to standard procedures [20]. Flowers were tagged at anthesis and fruits were harvested 25, 35, 45, 55 or 65 days later. Only flowers that formed on the main stem were permitted to set fruit; secondary flowers were pinched out as they formed.

After each harvest, fruits were transferred to the laboratory and randomly separated into three groups: (a) fruits were quartered on the same day and seeds extracted by the exertion of pressure under water, (b) fruits were stored for 20 days at room temperature (25±3^{0}C) before extracting seeds by the same procedure, (c) fruits were treated with ethylene prior to storage and seeds extracted as described in (a). Ethylene treatments were carried out by placing 6 fruits of each cultivar in a 34 l airtight container through the seal of which pure ethylene (Linde Hellas Industrial Gases, Schematar, Greece) was injected to achieve a concentration of 150 mg/l. The container remained sealed for 24 h after which the fruits were removed and stored in air for 20 days at room temperature (25±3^{0}C). Seeds were thoroughly washed in running tap water, followed by rinsing in deionised water and drying at 25^{0}C for 3 days. Seeds

were then counted, weighed and seed size estimated as the mean weight per 1000 seeds. Seeds were subjected to germination tests on the day they were weighed, i.e. 3 days after extraction. The seed tests were uniform for all seed lots. Because many of the seed lots germinated only slowly (largely due to immaturity) the germination test was extended beyond the time of counts set by the ISTA [1]. Tests were performed by placing four replicates of 50 seeds on absorbent paper in 8 cm Petri dishes. The paper was moistened with distilled water and the closed dishes incubated in the dark at 25±0.5^{0}C for 25 days. The temperature was that employed in earlier publications on the same species [11]. Germination was recorded at 1-2 day intervals and the germinated seeds were discarded. The criterion for germination was the appearance of the radicle with a length >2 mm. The results are presented as the total percent germination and the rate of germination (T_{50}), which is the time required for the germination of 50% of the total number of seeds that germinated in each test.

The experiment was repeated in year 2 for cv. Emi and Tsakoniki using the same cultivars as in year 1 except that fruits were harvested only at 25-45 days after anthesis (DAA) since the results of year 1 showed that this is the stage at which seed after-ripening is most beneficial. In year 2, sowing was carried out on 21 March and plants transferred to the greenhouse on 14 May. Seeds were extracted from fruits subjected to the same treatments as in year 1 but with the addition of an extra treatment in which fruit stored at room temperature (25±3^{0}C) before seed extraction were exposed to 100 mg/l ethylene for 24 h on days 1, 7 and 15 after harvest. Ethylene treatments were repeated three times as described above and the results are expressed as the means of the three experiments.

Data were subjected to analysis of variance and where the F value indicated significance, means were separated by the least significant difference test at p=0.05 using the statistical programme Statgraphics 2.1 (Statistical Graphics Corp., Herndon VA, USA).

RESULTS

The presence of two developing fruit on the mother plant of Emi led to a 30% reduction in flower induction compared with plants that were not permitted to set fruit, while in Black Beauty, Tsakoniki and Long Negro the corresponding inhibition was 55, 67 and 78% respecttively (Fig. 1). In Emi flower size over the same period was reduced by 3% due to the presence of two fruit on the mother plant, but in Long Negro, Tsakoniki and Black Beauty the corresponding reduction in flower size was 15, 21 and 21% respectively (Fig. 2).

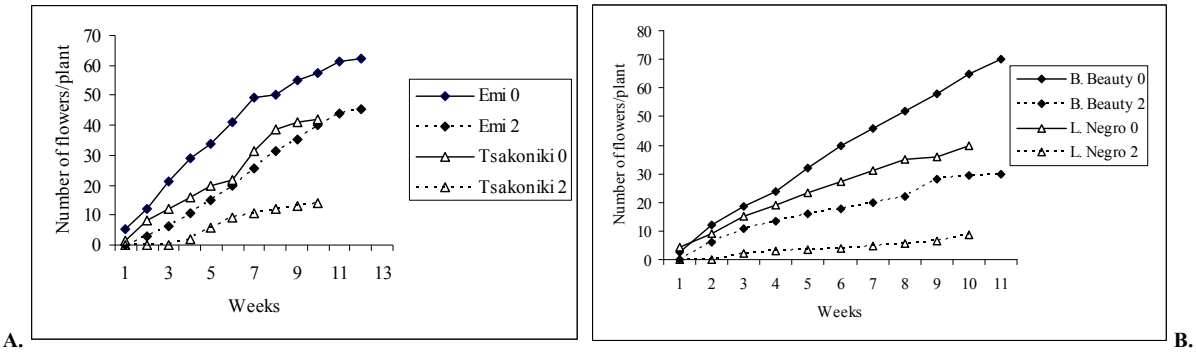

Figure 1. The effect of fruit set on subsequent flower induction of (**A**) Emi and Tsakoniki. and (**B**) Black Beauty and Long Negro. 0 = no fruit permitted to set per plant, 2 = 2 fruit set and permitted to set and mature on the plant.

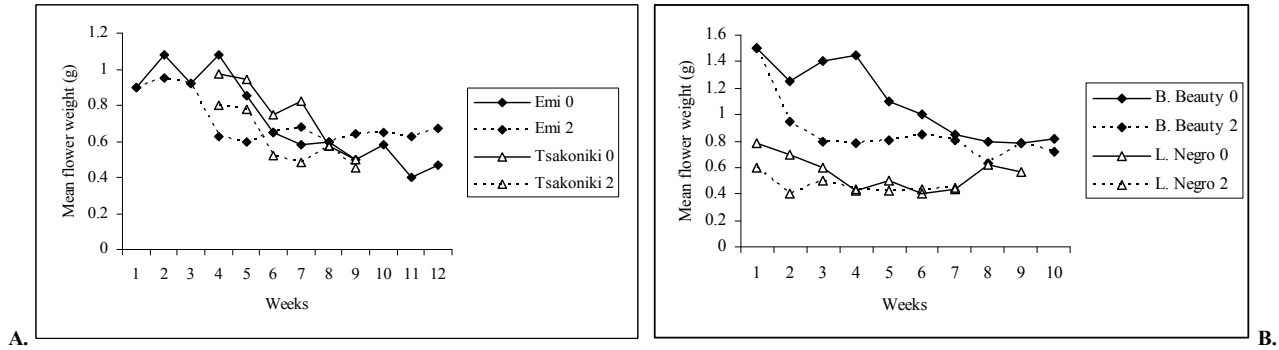

Figure 2. The effect of fruit set on mean flower weight of (**A**) Emi and Tsakoniki and (**B**) Black Beauty and Long Negro. 0 = no fruit permitted to set per plant, 2 = 2 fruit set and permitted to mature on the plant.

When fruit were harvested 25-35 DAA the seeds of all four cultivars were small and immature, with a mean 1000 seed weight of 1.0-2.4 g (25 DAA) and 1.2-3.8 g (35 DAA). At 45 DAA, mean 1000 seed weight rose to 4.0-5.3 g while at normal harvest time (55 DAA) it was 5.4-6.0 g (Table 1). When seeds were after-ripened within the harvested fruit for 20 days prior to extraction, seed size generally increased in the case of fruit harvested 25 or 35 DAA (except in Long Negro), but not at 45-55 DAA (all cultivars). Treatment of fruit with ethylene prior to the after-ripening procedure, however, apparently had no affect on mean 1000 seed weight

(Table 1). It is clear from this result therefore that although eggplant is a non-climacteric fruit, the seeds within the harvested fruit continue to fill and mature prior to extraction (i.e. during after-ripening), but ethylene does not promote this procedure.

Table 1. The effect of after-ripening with or without exposure to ethylene on the mean 1000 seed weight (g) of four eggplant cultivars harvested at 25-55 days after anthesis.

Cultivar	Treatment	Days from anthesis to harvest			
		25	35	45	55
Black Beauty	None	1.1 c*	2.2 b	4.8 a	6.0 a
	After-ripened	3.2 a	4.6 a	4.8 a	5.4 a
	A-r + ethylene	2.1 b	3.8 a	5.2 a	5.6 a
Long Negro	None	2.4 a	3.8 a	4.0 a	5.4 a
	After-ripened	1.8 a	2.4 b	4.5 a	4.9 a
	A-r + ethylene	1.7 a	3.7 a	4.0 a	5.2 a
Tsakoniki	None	1.0 b	1.2 c	5.3 a	5.5 b
	After-ripened	1.4 ab	2.8 b	5.3 a	6.2 a
	A-r + ethylene	1.7 a	3.7 a	5.0 a	6.6 a
Emi	None	1.4 b	3.0 a	5.1 a	5.6 a
	After-ripened	2.3 a	3.3 a	5.0 a	5.9 a
	A-r + ethylene	2.6 a	4.2 a	4.8 a	5.8 a

* Means within columns for each cultivar followed by the same letter do not differ significantly at p=0.05.

Seeds extracted from fruit that were harvested 25 DAA failed to germinate irrespective of cultivar. When harvest was carried out 35 DAA, seeds of Emi and Tsakoniki again failed to germinate, whereas seeds of Long Negro and Black Beauty germinated to a level of 11.5 and 20% respectively. At 45 DAA the germination of all cultivars increased significantly and at 55 DAA was higher than 95% in all cases (Table 2). Although after-ripening of seed prior to extraction from fruits harvested 25-35 DAA induced an increase in the germination of Emi and Tsakoniki, it had little or no effect on Long Negro and Black Beauty. The exposure of fruit to ethylene prior to the after-ripening procedure had a variable effect on seed germination. In Long Negro and Black Beauty, ethylene treatment promoted the germination of seeds extracted from fruit harvested at 25 and 35 DAA to a level beyond that induced by after-ripening, but in Emi and Tsakoniki ethylene treatment significantly reduced the efficacy of the after-ripening process (Table 2). Overall, the rate of germination (T_{50}) of seeds extracted from fruit of all four cultivars harvested 45 or 55 DAA ranged between 14-18 days and 14-15 days respectively. After-ripening increased the rate of germination, as shown by a reduction in the value T_{50} to 6-12 days (45 DAA) and 8-11 days (55 DAA), irrespective of ethylene application.

Table 2. Effect of after-ripening and ethylene treatment on the germination (%) of eggplant seeds measured 3 days after seed extraction (year 1).

Cultivar	Treatment	Days from anthesis to harvest			
		25	35	45	55
Black Beauty	None	0 c*	11.5 b	91.5 a	100 a
	After-ripened	5.5 b	9.5 b	97.0 a	98.0 a
	A-r + ethylene	34.5 a	41.0 a	96.0 a	92.0 a
Long Negro	None	0 c	20.0 b	46.0 b	95.5 b
	After-ripened	18.0 b	24.0 b	99.0 a	100 a
	A-r + ethylene	56.0 a	80.5 a	100 a	86.0 c
Tsakoniki	None	0 c	0 c	85.5 b	100 a
	After-ripened	43.5 a	96.5 a	100 a	100 a
	A-r + ethylene	28.0 b	2.0 b	31.0 c	42.0 b
Emi	None	0 c	0 c	70.5 b	97.5 a
	After-ripened	97.0 a	100 a	97.5 a	100 a
	A-r + ethylene	12.5 b	11.5 b	17.0 c	27.5 b

* Means within columns for each cultivar followed by the same letter do not differ significantly at p=0.05.

Because Emi and Tsakoniki are particularly important local cultivars produced exclusively from local seed, the value of increasing the exposure of fruit to ethylene during the after-ripening procedure was examined in year 2. As in year 1, after-ripening promoted the germination of seeds of both cultivars extracted from fruit harvested at 25 and 35 DAA, as well as Emi at 45 DAA (Table 3). The action of ethylene, however, was again variable. A single exposure to ethylene promoted the germination of Tsakoniki and Emi harvested 35 DAA, but not 25 or 45 DAA. A triple exposure of fruit to ethylene further increased the germination of Tsakoniki harvested 35 DAA, but had no effect in the other cases (Table 3).

The rate of germination (T_{50}) of seeds extracted from fruits harvested 35 DAA following after-ripening varied between 11 and 20 days and was not affected by ethylene treatment. At 45 DAA, the T_{50} of seeds from after-ripened fruit was 11-24 days (a rate not affected by ethylene treatment) compared with 13-19 days for seed from fruit without after-ripening. The T_{50} of seed from fruit harvested at 25 DAA (also at 35 DAA in the absence of after-ripening) could not be assessed due to insufficient germination.

Table 3. Effect of after-ripening and ethylene treatment on the germination (%) of eggplant seeds measured 3 days after seed extraction (year 2).

Cultivar	Treatment	Days from anthesis to harvest		
		25	35	45
Tsakoniki	None	0 b*	0 d	98.0 a
	After-ripened	23.5 a	49.8 c	93.7 a
	A-r + ethylene 1	23.2 a	69.3 b	92.8 a
	A-r + ethylene 3	19.3 a	84.8 a	ND
Emi	None	0 c	15.0 c	70.0 b
	After-ripened	41.5 a	51.4 b	91.8 a
	A-r + ethylene 1	16.2 b	65.5 a	94.6 a
	A-r + ethylene 3	49.2 a	72.3 a	ND

* Means within columns for each cultivar followed by the same letter do not differ significantly at p=0.05. ND = not determined.

DISCUSSIONS

For seed production eggplant fruits should be harvested at a later stage of ripeness than those for fresh consumption [7]. Thus the optimum harvest time for eggplant seed is 50-60 DAA in Turkey [5] and S.E. Asia [3, 18, 21]. However, during fruit growth and maturation there is competition between fruits on the plant for essential nutrients and storage reserves, which leads to a reduction in flower induction (Fig. 1) and flower size (Fig. 2) [12] and may adversely affect seed yield. The effect of fruit load on subsequent flowering and flower size varied between cultivars. Flower induction in Black Beauty and Emi, which produce large, flask-shaped fruit, was less sensitive to fruit load than in Tsakoniki and Long Negro, which produce elongate fruit. Moreover, the reduction in flower size was less in Emi than in the other three cultivars. Emi has the ability to set fruit with few or no seeds whereas both Tsakoniki and Long Negro usually set fruit only when the number of seeds formed is high [14].

When fruits were harvested at 25-35 DAA, seeds were small (Table 1) and either did not germinate at all or only to a very low level (Table 2). Demir *et al.* [5] reported that seed filling in a field-grown eggplant cultivar "Pala" in Turkey took 40-42 DAA, whereas maximum seed quality was attained 10-20 days later. Moreover, the mean 1000 seed weight for eggplant is about 5 g [7], except for some very small seeded cultivars. In the present experiment, a mean 1000 seed weight of over 5 g was observed in Emi and Tsakoniki at 45 DAA, although seed continued to fill until 55 DAA. Seed of Black Beauty and Long Negro attained a 1000 seed weight of over 5 g at 55 DAA, indicating cultivar differences in the rate of filling (Table 1). Yogeesha *et al.* [21] found germination to be negligible before 41 DAA, but both germination and vigour were maximal at 57 DAA, which was the stage at which fruit started to turn brown. Under the conditions of the present experiments and in the absence of after-ripening, we recommend that fruit should ripen on the plant for at least 55 DAA in order to achieve good seed quality.

When eggplant fruit are harvested prior to maturity (25-35 DAA) and held for 20 days at room temperature (25±3^{o}C) prior to seed extraction, the seed continue to fill and mature within the fruit as indicated by the relative increase in mean 1000 seed weight (except Long Negro) and the increase in percent germination (all cultivars). This result confirms the observations of a beneficial effect of after-ripening on eggplant cultivars Emi and Tsakoniki [15] and extends the results to Black Beauty and Long Negro. For fruit harvested at 45-55 DAA the seed was generally mature, with a high level of germination that was not further increased by after-ripening (except Long Negro and Emi 45 DAA) indicating that by this stage filling and maturation of seeds was complete [5].

Eggplant is a non-climacteric fruit and therefore ripening is not induced by exposure to ethylene [13]. The fact that seeds continue to fill and ripen within the harvested fruit, however, suggests that seed development occurs independently of fruit ripening [15]. The variable effect of ethylene on the germination of seed extracted from immature fruit (Table 2) indicates a clear difference between cultivars. The seed of Black Beauty and Long Negro harvested 25 or 35 DAA responded positively to a single application of ethylene while those of Emi and Tsakoniki did not (Table 2). It may, however, be that Emi and Tsakoniki are less sensitive to ethylene because in the second experiment ethylene had a positive effect on Emi and Tsakoniki harvested 35 DAA and stored for 20 days prior to seed extraction and this effect was higher in both cultivars when ethylene was applied three times during the storage (after-ripening) period (Table 3).

In conclusion, it is clear from the present results that if eggplant fruits are harvested prior to maturity (i.e. 25-35 DAA) and stored at 25^{o}C for 20 days prior to seed extraction, the seeds within the fruit continue to fill and mature (after-ripen) and acquire the ability to germinate. This suggests a possible application of after-ripening to reduce the duration of fruit maturation on the mother plant during which time the fertility of subsequently formed flowers may be adversely affected, thus restricting fruit set and yield [8, 12, 19]. However, the success of this procedure is greatly influenced by the cultivar, perhaps due to different rates of seed filling and maturation. Although harvest 35 DAA coupled with after-ripening results in seed with a high level of germination in Emi and Tsakoniki, this does not apply to Long Negro and Black Beauty; moreover harvest of all cultivars at 25 DAA appears too early. The treatment of prematurely harvested fruit with ethylene does not affect seed size, but may improve germination ability. However, response to ethylene treatment varies between cultivars, indicating a need for further research in this area.

REFERENCES

[1] Anonymous, (1985): International rules for seed testing. Seed Science and Technology, 13: 299-355.

[2] Bebeli, P.J., Mazzucato, A., (2008): The Solanaceae – a review of recent research on genetic resources and

advances in the breeding of tomato, pepper and eggplant. European Journal of Plant Science and Biotechnology, 2(1): 3-30.
[3] Chen, N.C., (2001): Eggplant seed production. AVRDC International Cooperators' Guide. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 4.
[4] Daunay, M-C., Janick, J., (2007): History and iconography of eggplant. Chronica Horticulturae, 47: 16-22.
[5] Demir, I., Mavi, K., Sermenli, T., Ozcoban, M., (2002): Seed development and maturation in aubergine (*Solanum melongena* L.). Gartenbauwissenschaft, 67: 148-154.
[6] Demir, K., Bakir, M., Sarıkamış, G., Acunalp, S., (2010): Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. Genetics and Molecular Research, 9(3): 1568-1576.
[7] George, R.A.T., (1985): Vegetable seed production, 1st ed. Longman, London, UK, pp. 318.
[8] Lenz, F., (1970): Effect of fruit on sex expression in eggplant (*Solanum melongena* L.) varieties with reference to weather conditions. Horticultural Research, 10: 81-82.
[9] Muñoz-Falcón, J.E., Prohens, J., Rodriguez-Burruezo, A., Nuez, F., (2008): Potential of local varieties and their hybrids for the improvement of eggplant production in the open field and greenhouse cultivation. Journal of Food, Agricultural and Environment, 6(1): 83-88.
[10] Muñoz-Falcón, J.E., Prohens, J., Vilanova, S., Nuez, F., (2008): Characterization, diversity, and relationships of the Spanish striped (Listada) eggplants: a model for the enhancement and protection of local heirlooms. Euphytica, 164: 405-419.
[11] Passam, H.C., Akoumianakis, K., Khah, E.M., (1999): Long-term storage of aubergine seed following production under high ambient temperatures. Seed Science and Technology, 27: 977-980.
[12] Passam, H.C., Baltas, C., Boyiatzoglou, A., Khah, E.M., (2001): Flower morphology and number of aubergine (*Solanum melongena* L.) in relation to fruit load and auxin application. Scientia Horticulturae, 89: 309-316.
[13] Passam, H.C., Karapanos, I.C., (2008): Eggplants, peppers and tomatoes: factors affecting the quality and storage life of fresh and fresh-cut (minimally-processed) produce. European Journal of Plant Science and Biotechnology, 2 (Special issue 1): 156-170.
[14] Passam, H.C., Khah, E.M., (1992): Flowering, fruit set and fruit and seed development in two cultivars of aubergine *(Solanum melongena* L.) grown under plastic cover. Scientia Horticulturae, 51: 179-185.
[15] Passam, H.C., Theodoropoulou, S., Karanissa, T., Karapanos, I.C., (2010): Influence of harvest time and after-ripening on the seed quality of eggplant. Scientia Horticulturae, 125: 518–520.
[16] Petrov, H., Doikova, M., Popova, D., (1981): Studies on the quality of eggplant seed. Acta Horticulturae, 111: 273-280.
[17] Prohens, J., Blanca, J.M., Nuez, F., (2005): Morphological and molecular variation in a collection of eggplant from a secondary center of diversity: implications for conservation and breeding. Journal of the American Society for Horticultural Science, 130: 54–63.
[18] Rashid, M.A., Singh, D.P., (2000): A manual on vegetable seed production in Bangladesh. AVRDC-USAID-Bangladesh Project, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Bangladesh, pp. 119.
[19] Sekara, A., Bieniasz, M., (2008): Pollination, fertilization and fruit formation in eggplant (*Solanum melongena* L.). Acta Agrobotanica, 61: 107-113.
[20] Smith, D., (1979): Peppers and aubergines. Grower Guide 3. Grower Books, London, UK, pp. 91.
[21] Yogeesha, H.S., Singh, T.H., Naik, L.B., (2008): Seed germination in relation to seed development in eggplant (*Solanum melongena*). Indian Journal of Agricultural Science, 78: 1010-1012.

Submitted: 9 September 2010
Accepted: 2 October 2010
Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433