Vegetable Science (2020) 47(2): 266-273

Physio-biochemical changes associated with spatial differences of ovules in cucumber (*Cucumis sativus* L) fruit under open field and protected environments

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Received: July 2020/ Accepted: September 2020

Abstract

Seeds from middle and stylar fruit segments showed superior performance in terms of seed yield and quality in chosen two cucumber varieties (Pusa Barkha and Pusa Uday) under both open field (E1) and protected (E2) environments, during 2019-20, at ICAR-Indian Agricultural Research Institute, New Delhi. Besides, both seed quality and yield were superior under protected environment (E2). This may be due to temporal and spatial advantage of stylar segment in receiving the vigorous pollen and garnering higher food accumulates (protein, sugar, starch and oil) than peduncular segment; thus, higher probability of seed setting. Higher dehydrogenases, antioxidants (SOD, CAT & POX) and lower ROS (H_2O_2 & O_2^{-}), electrical conductance, TSS and TSP (seed leachates) from middle and stylar segment of fruit indicated the better physical and physiological soundness of seed. The seeds from peduncular segment lagged behind in development and maturation, showed higher dormancy. Therefore, to obtain optimum seed quality in cucumber, seed should be harvested from middle and stylar segments of fruit.

Keywords: Cucumber, ovule position, ROS, antioxidants, seed composition, seed quality & yield, protected environment

Introduction

Higher number of underdeveloped seeds is a major problem in cucumber (*Cucumis sativus* L) seed production. Seeds are vital and nutrient-dense part of fruit, which contain phytonutrients, fibres and antioxidants that help in human immunity, skin, eye and prevention of cancer (Abbey et al. 2017). Among various extrinsic and intrinsic factors *viz* genotype, growing environment (soil, climate etc), cultural practices and stage of seed harvest (Delouche 1980), the seed position on mother plant is a major (intrinsic) factor, which affects seed quality and yield to a greater extent. The position of seed is determined by the position of the ovules within the fruit, and fruit position on the mother plant. Many reports have shown that seed performance differs largely among seeds collected from different segments of fruit in ash gourd (Murugesan and Vanangamudi 2005); pumpkin (Katuboina 2012); pod of *Bauhinia ungulate* (Mena-Ali and Rocha 2005); zones of sunflower capitulum (Shekhargouda et al. 1996); umbels of carrot (*Daucus carota* L) Corbineau et al. (1995) and *Peucedanum oreoselinum* (Ko³odziejek 2017).

Jing et al. (2000), in cucumber, reported that seed maturation; attainment of dry weight and desiccation tolerance differs in seeds from peduncular segment than those from other fruit segments in temperate cucumber, probably due to differential cell cycle in terms of nuclear DNA, â-tubulin and seed chlorophyll fluorescence. Several morphological, physiological and biochemical changes (*viz* seed composition, ROS and antioxidants) associated with ovule position in fruit in Indian cucumber are yet to be elucidated, as well the regulation of seed dormancy. The position of seeds on the parental plant could have differential seed dormancy (Lu *et al*, 2017). Jing et al. (2000) and Gupta et al. (2020 personal communication), reported the presence of primary dormancy in developing cucumber seed; Aroonrungsikul et al. (1997) and Patil (2018) reported the role of intrinsic ABA and some other germination inhibitors from seed coat. The seeds of cucumber being impregnated in the pulpy mass of fruit (fully hydrated environment) during the entire period of development, the risk of breakdown of dormancy and viviparous germination is also

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associated. Under hydrated condition of seed, the ROS activity was reported to be increased in mitochondria and plasma membrane by NADPH oxidase (Leubner-metzger 2005), favouring the germination. These ROS under the strict supervision of antioxidants regulates the ABA/GA ratio (Bailly 2019), consequently decides the fate of seed germination. Hence, necessitated to investigate the ROS and certain antioxidants in seeds of cucumber from various positions in fruit. The assessment of antioxidants in seed also became important as seeds are used in cosmetics and prevention of cancer. Therefore, the present study was aimed to determine the physiological and biochemical changes associated with spatial differences of seed in cucumber fruit from chosen two varieties Pusa Barkha and Pusa Uday under open field and protected environments.

Materials and Methods

Experimental materials and site: Two cucumber varieties *viz*, Pusa Barkha and Pusa Uday (procured from Division of Vegetable Science, ICAR– Indian Agricultural Research Institute, New Delhi (IARI)), were chosen and studied under two environments (i) open field (E1 - at research farm of Division of Vegetable Science), and (ii) protected environment (E2 -at Centre for Protection Cultivation Technology), in IARI. Total thirty plants (10 in 3 replicates in 3 separate plots (size $6.75m^2$)) of each variety were grown on raised beds under two seasons (*viz,* summer-2019 & *kharif*-2019 under E1, and summer-2019 & winter-2019 under E2). Five to ten flowers, from each vine were hand pollinated (7-10 am) and tagged. The fruits were harvested, from nearly similar nodes (to nullify the differences due to age and position), on 45-days from pollination (DFP) under E1, whereas on 60 DFP under E2. Fruits were divided into three equal parts namely, stylar, middle and peduncular segments; seeds from these were collected separately and stored for 45 days at laboratory ambient temperature (as fresh seeds exhibit dormancy) before use. Seed quality parameters *viz,* seed germination and moisture content were measured following ISTA rules (Anon 2019), whereas seed vigour indices following Abdul-baki and Anderson (1973).

Physiological and biochemical assays: To measure electrical conductance (EC), ten seeds (pre-weighed) in three replicates were soaked in 30 ml MilliQ water (MW) (Millipore, Gradient SAS–67, France) and allowed to stand at 25°C for 24 hrs. EC was measured using a digital conductivity meter (CM 183, Elico, India) and expressed as $\mu S\ cm^{-1}g^{-1}$ (Pandita and Nagarajan 2006). Total soluble sugars (TSS) and proteins (TSP) from seed leachates with different treatments were measured following Dubois et al. (1956) and Bradford (1976), respectively. Starch content in seeds was estimated using Anthrone method (Hodge and Hofreiter 1962). Seed oil content (2g) was determined using a Soxhlet apparatus (AOAC 1990). Superoxide anion ($O_2^{\cdot-}$) (0.5g seed) was measured (NBT reduction) and expressed as $ÄA_{540}\ min^{-1}g^{-1}_{FW}$ following Chaithanya and Naithani (1994). H_2O_2 was estimated by the (formation of titanium-hydro peroxide and expressed as $\mu mol\ H_2O_2\ g^{-1}_{fw}$ (Mukherjee and Choudhari 1983). The dehydrogenases activity using 15 seeds in three replication each was measured following (Kittock and Law 1968). SOD was assayed (inhibition of photochemical reduction of NBT) following Dhindsa et al. (1981). Activity of catalase was measured (quantifying the residual H_2O_2 in the reaction mixture) and expressed as ìmol $g^{-1}_{fw}\ min^{-1}$ (Aebi et al. 1984). POX was assayed (formation of tetraguaiacol (Î= 26.6mM^{-1}cm^{-1}) and expressed as µmol tetra-guaiacol formed $min^{-1}g^{-1}_{fw}$ (Rao et al. 1996).

The experiment was conducted following CRBD in three replicates, in a bifactorial scheme and subjected to ANOVA. The germination percentages were transformed as arc sine values, to normalize the data, before statistical analysis. Statistical analyses were carried out using WASP 2.0; graphs were generated using Microsoft Excel 2019; graph tool and 'R' statistical packages.

Results and Discussion

Seed yield: The significant differences in seed yield under two growing environments were recorded. This may be attributed to optimum growing conditions; relatively longer duration of maturity; with reduced biotic and abiotic stresses; better seed development and maturation under protected environment (E2) than those in open field environment (E1). Irrespective of varieties and seasons, the differences were significant in total seed numbers and/or filled seeds extracted from different fruit segments. The seeds from the middle segment had maximum number of seeds (154.5 & 159.0), number of filled seeds (122.0 (80.92%) & 131.5 (82.68%), whereas the minimum was recorded from peduncular segment [53.0 (58.34%) & 52.8 (57.81%)] under E1 and E2, respectively (Fig 1). The higher number of filled seed in middle and stylar segments could be attributed to reduced probability of being unfertilized or seed abortion at an early and later developmental stage, which may be a consequence of the gametophytic competition for the access to ovules as well as resource limitation (Mena-Ali and Rocha 2005; Rocha and Stephens 1991). It is presumed that ovules located closer to the point of entry of pollen tubes will present a lower probability of abortion, whereas those found farther from the entry point will present a higher probability of abortion (Mohan Raju et al.1996, Kumar et al. 2015). Thus, seeds from advantageous ovule positions (eg the stylar and middle segment of a fruit) exhibit higher seed dry weight and quality, whereas seeds from disadvantaged positions (*eg*. the peduncular end of a fruit) either exhibit low quality or fail to reach maturity.

Results showed the maximal seed dry weight was observed from middle (25.8 & 26.5mg seed^{-1}) followed by stylar (23.8 & 24.2mg seed^{-1}) under E1 and E2, respectively (Fig 2). However, the differences were non-significant between two environments. Likewise, 1000-seed weight was maximum in seeds from middle (25.82 and 26.98g) followed by stylar (24.55 and 25.48 g) under E1 and E2, respectively (Fig 2). Variation in seed dry weight may also be attributed to higher amount of assimilates, the major food reserves namely, TSS, TSP, total starch and oil contents (Fig 3). Irrespective of varieties and seasons, maximal values for TSS (3.027&3.143mg g^{-1}_{dw}); TSP (175.14 & 205.35mg $g^{-1}_{dw)}$; total starch (22.38 & 22.91mg g^{-1}_{dw}) and oil contents (30.37 & 31.89%) were observed from middle segment followed by stylar segment, whereas the minimum values from peduncular segment under E1 & E2, respectively (Fig 3). The differences in seed composition (food reserves *ie,* TSS, TSP and total starch contents) between middle and stylar segments were non-significant except for oil contents, which was (1.15-folds) more in seeds from stylar segment. The differences for food reserves in seeds from middle segment were significantly superior from peduncular segment *viz* TSS (1.31-folds & 1.30-folds); TSP (1.17-folds & 1.30-folds), total starch (1.29-folds & 1.28-folds) and oil contents (1.40-folds & 1.43-folds) under E1 and E2, respectively.

On the other hand, cylindrical shape of fruit favours the development of seed and proper supply of assimilates (wider area of middle segment than those to narrower end of peduncular segment). The significantly different performance of seeds from three fruit segments indicated that seeds within a fruit (from different segments) may not be of the similar age, as they do not mature at the same rate and time (Nielsen 1996). This is probably due to pollen grain germination, pollen tube growth, zygote formation, seed development and maturation, which may not occur at the same rate and time (Delph et al. 1998).

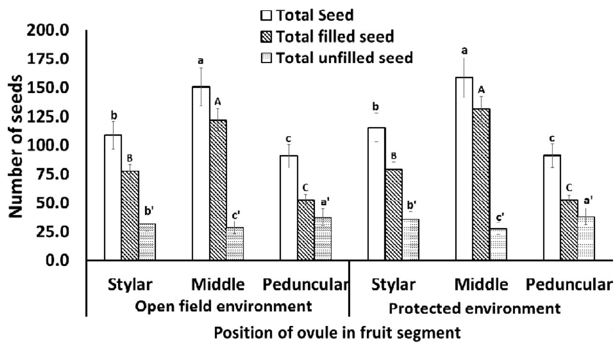
Seed quality: The variations in seed quality from three segments of fruit were significant. The seeds from middle and stylar segments were superior in respect of seed germination and vigour indices. Irrespective of varieties and seasons, the maximal mean germination values were recorded from middle (76.9 & 79.2%), followed by stylar (73.8 & 75.8%), whereas the minimum from peduncular segments (66.6 & 69.1%) under E1 and E2, respectively (Fig 4). Likewise, seed vigour indices *viz* seed vigour index-I [derived using germination(%) and seedling length(cm)] were significantly higher in middle (1289.4 &1434.2) followed by stylar (1199.1 & 1350.8) and least in peduncular segments (958.6 &1181.9); seed vigour index-II [derived using germination(%) and seedling dry weight(g 10 seedling^{-1}] were higher in middle (9.67 & 10.65) followed by stylar (8.94 & 9.89), whereas least in peduncular segments (7.43 & 8.56) under E1 and E2, respectively (Fig 4). The differences in seed germination and vigour could also be attributed to vigour differences in pollen, which participating in pollination and differential transfer & accumulation of food reserves in seed.

TSS and TSP from seed leachates were significantly different in seed obtained from all three fruit segments. Irrespective of varieties and seasons, the minimum EC was recorded from middle segment (107.1 & 93.6μScm^{-1}g^{-1}), whereas maximum from peduncular segment (149.8 & 132.8μScm^{-1}g^{-1}) under E1 and E2, respectively (Fig 5). Minimum TSS (seed leachate) were recorded in seeds from middle segment with the mean values (0.772 & 0.760mg g^{-1}) followed by stylar segment (0.922 & 0.901mgg^{-1}), whereas maximum from peduncular segment (1.054 &1.028mg g^{-1}) under E1 &

Table 1: Effects of ovule position in fruit on seed viability and germination in cucumber grown under two environments

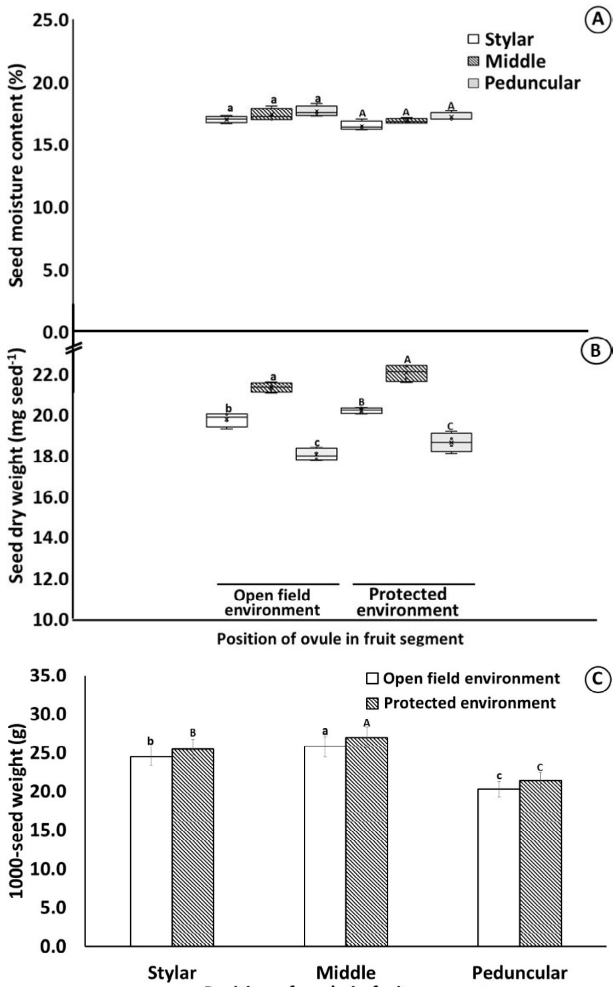
Fruit segment	Environment							
	Open field (E1)				Protected (E2)			
	Viability of fresh seeds (%)	Germination of fresh seeds (%)	Germination of45-days stored seeds (%)	Germination of GA (0.02%) treated seeds (%)	Viability of fresh seeds (%)	Germination of fresh seeds (%)	Germination of 45-days stored seeds (%)	Germination of GA (0.02%) treated seeds (%)
Stylar	83.18	8.00	71.25	73.79	84.2	9.00	74.33	75.79
Middle	84.29	9.00	72.75	76.94	85.43	11.00	76.50	79.21
Peduncular	80.58	0.00	65.33	66.63	82.33	0.00	67.33	69.06
CD@5%	2.17	-	4.10	3.88	1.99	-	3.75	4.02
CV%	4.34	-	5.29	6.56	5.38	-	4.88	5.99

Note: Values are mean of two varieties from two seasons; Values in %ages were arc sin transformed prior to statistical analysis to normalize the data



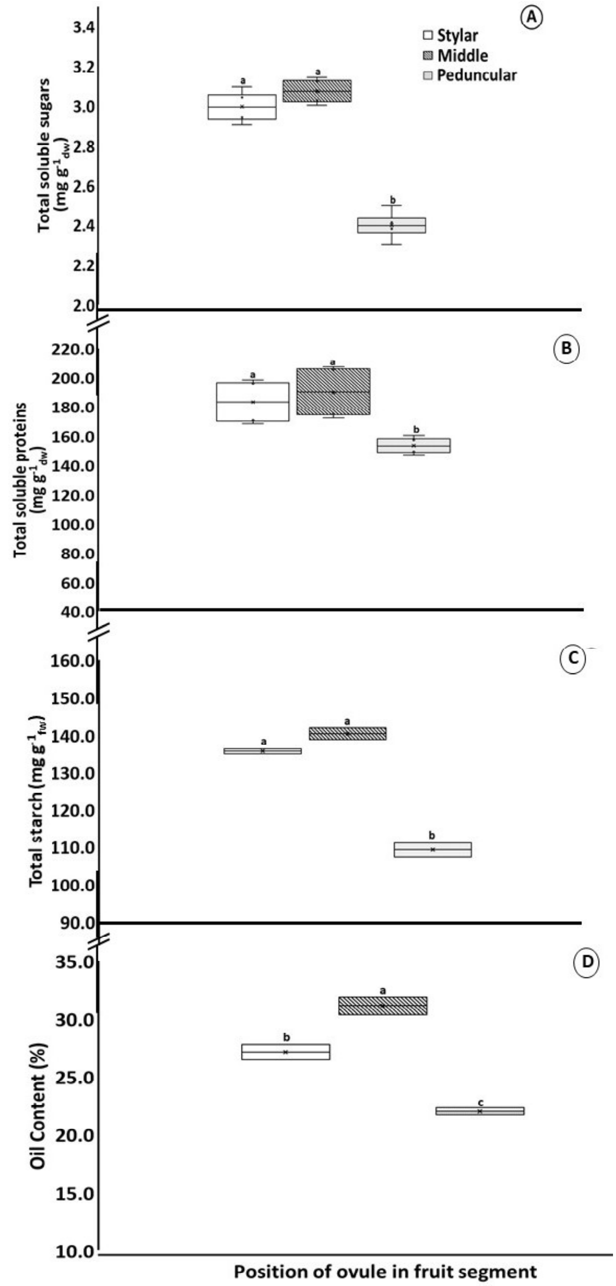
(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd"0.05 following Duncan's multiple range test).

Fig1: Effects of position of ovule in different fruit segments on number of seeds across cucumber varieties, seasons and environments



(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd"0.05 following Duncan's multiple range test).
Where, (A): Seed moisture content; (B): Seed dry weight and (C): 1000-seed weight

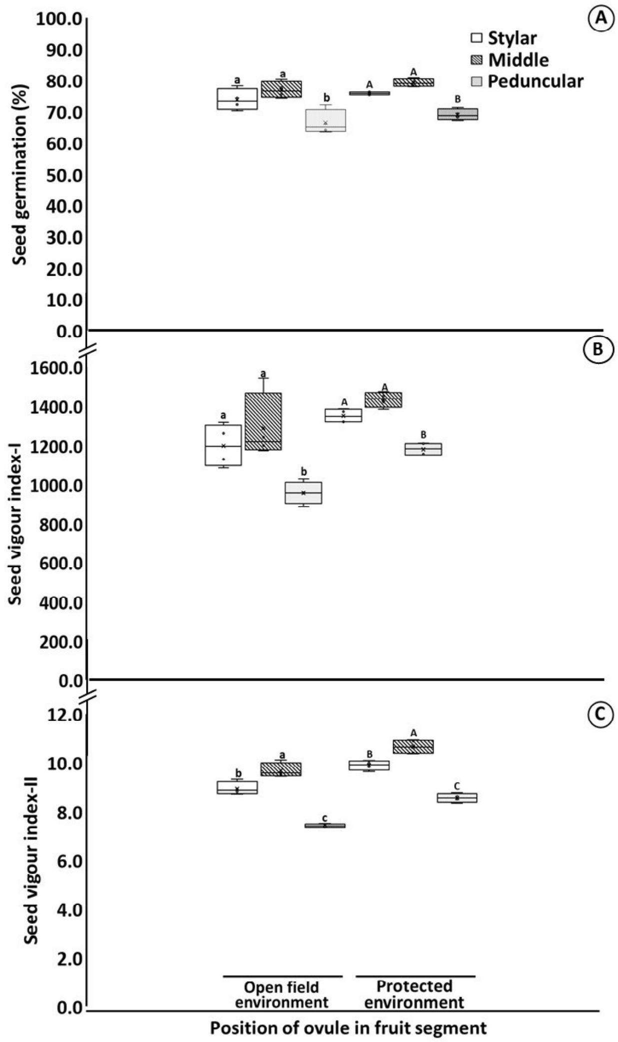
Fig 2: Effects of position of ovule in different fruit segments on seed moisture content, seed dry weight and 1000-seed weight across cucumber varieties, seasons and environments



(Data represent the mean values±SE of two varieties from two seasons using three replicates; Values denoted by different letters are significantly different at pd"0.05 following Duncan's multiple range test).Where, (A): Total soluble sugars; (B) Total soluble proteins; (C): Total starch and (D): Oil content

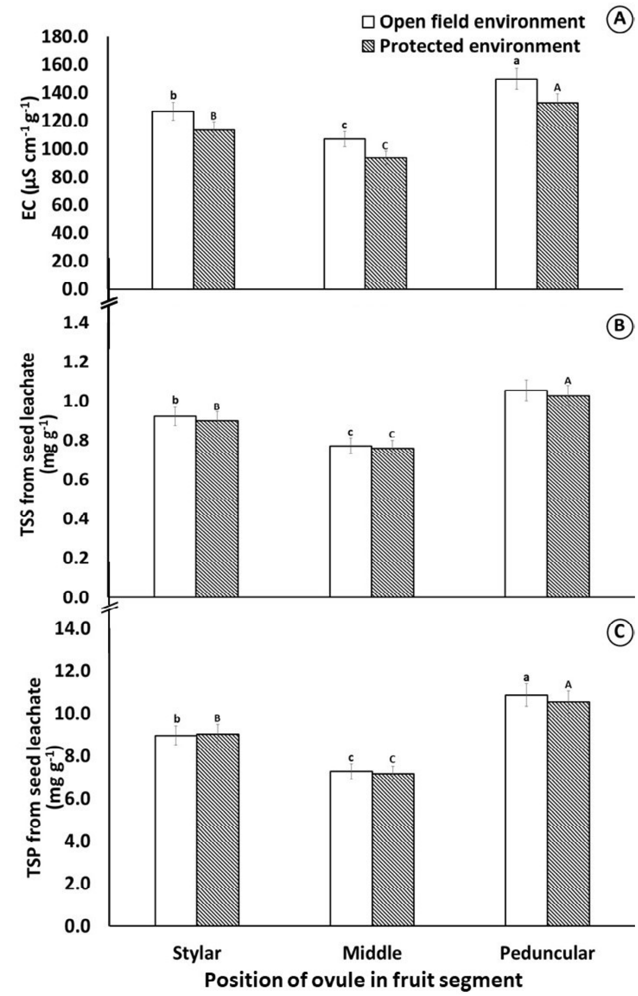
Fig 3: Effects of position of ovule in different fruit segments on seed composition across cucumber varieties, seasons and environments

E2, respectively (Fig 5). Likewise, minimum TSP (seed leachate) were from seed of middle segment with the mean values (7.26 & 7.15mg g^{-1}), followed by stylar segment (8.97 & 9.03mg g^{-1}), whereas the maximum from peduncular segment (10.8 & 10.5mg g^{-1}) under E1 & E2, respectively (Fig 5). Besides, the maximal dehydrogenases activity was recorded in seeds from middle segment (2.078 & 2.179µgg^{-1}), whereas it was



(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd"0.05 following Duncan's multiple range test).
Where, (A): Seed germination; (B): Seed vigour index-I and (C): Seed vigour index-II

Fig 4: Effects of position of ovule in different fruit segments on seed germination and vigour indices across cucumber varieties, seasons and environments

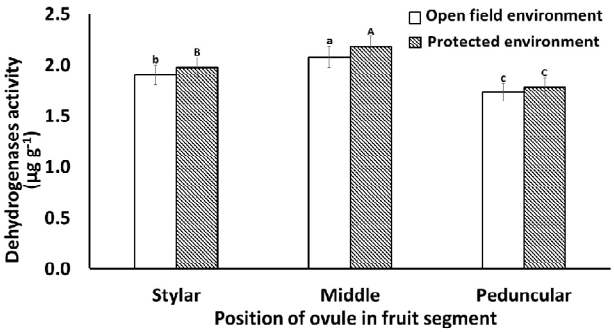


(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd"0.05 following Duncan's multiple range test). Where, (A): Seed electrical conductivity; (B): Total soluble sugars and (C): Total soluble proteins

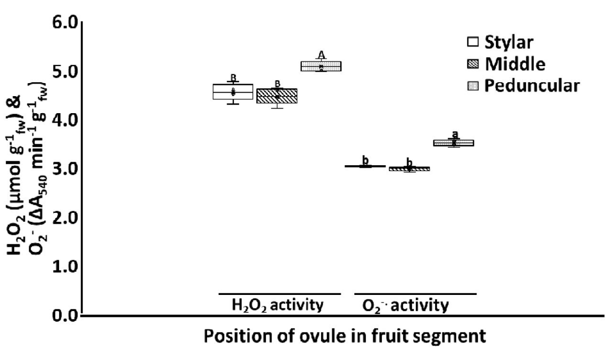
Fig 5: Effects of position of ovule in different fruit segments on electrical conductivity, total soluble sugars and total soluble proteins from seed leachates across cucumber varieties, seasons and environments

minimal from peduncular segment (1.734 & 1.784µgg^{-1}) under E1 & E2 environments, respectively (Fig 6). The higher values of seed vigour, from middle or stylar segments, indicated the physiological soundness of seed which could be due to higher dehydrogenases activity and lower EC, TSS & TSP (from seed leachates). The results were in conformity with Ko³odziejek (2017) in *Peucedanum oreoselinum*; Mena-ali and Rocha (2005) in *Bauhinia ungulate*; Murugesan and Vanagamudi (2005) in ash gourd; Jing et al. (2000) in cucumber; and Shekhargowda et al. (1996) in sunflower.

The seed moisture content among three segments of fruit varied between 16.44 to 17.65% (Fig 2). The differences were non-significant, irrespective of growing environments. Differences in seed dry weight due to differential seed filling, moisture content (non-significant), dehydrogenases activity and EC from seeds of a given fruit segment clearly showed the developmental variations. Seeds from stylar and middle segments developed early and faster, whereas the seeds from peduncular segment lagged behind. Jing et al. (2000) reported similar results in cucumber. The developmental variation attracts the attention towards the redox status of seeds, which is maintained by ROS and antioxidants. In present study, irrespective of varieties, growing seasons and environments, the ROS (H_2O_2 & $O_2^{\cdot-}$), in seeds collected from three segments of fruit, were maximal from peduncular followed by



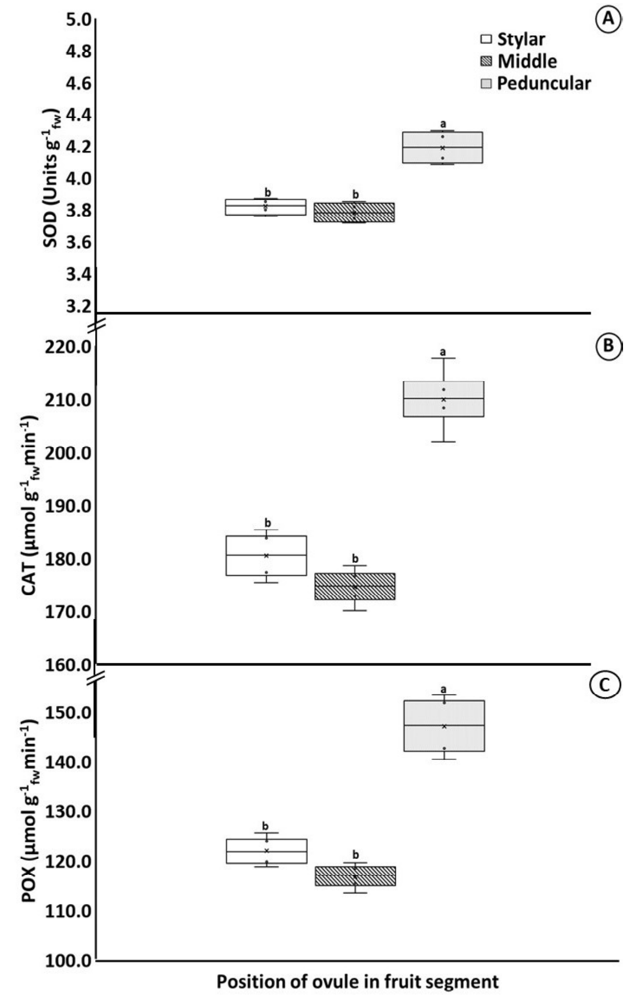
(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd”0.05 following Duncan's multiple range test)
Fig 6: Effects of position of ovule in different fruit segments on dehydrogenases from seed across cucumber varieties, seasons and environments



(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd”0.05 following Duncan's multiple range test)
Fig 7: Effects of position of ovule in different fruit segments on ROS activity from seed across cucumber varieties, seasons and environments

stylar and minimum from middle segments. Minimum values of H_2O_2 & $O_2^{\cdot-}$ were from middle segment (4.458 $\mu molg^{-1}_{fw}$ & 2.971 " $A_{540}min^{-1}g^{-1}_{fw}$), whereas the maximum values were from peduncular segment (5.079 $\mu molg^{-1}_{fw}$ & 3.509 " $A_{540}min^{-1}g^{-1}_{fw}$), respectively (Fig 7). The antioxidant enzymes (*viz* SOD, CAT, and POX), from three fruit segments, were in following order peducular<stylar< middle. The minimum values for SOD, CAT & POX were from middle segment (3.779 Units g^{-1}_{fw}, 174.5 µmol g^{-1}_{fw} min^{-1}& 116.7 µmol g^{-1}_{fw} min^{-1}), whereas maximum was in peduncular segment (4.189 Units g^{-1}_{fw}, 209.9 $\mu molg^{-1}_{fw}min^{-1}$& 147.0 $\mu molg^{-1}_{fw}min^{-1}$), respectively (Fig 8).

Differential ROS (H_2O_2 & $O_2^{\cdot-}$) and antioxidant enzymes activities showed that seeds from middle segment were physically and physiologically sound than those to peduncular segment of fruit. ROS generation is a continuous process during seed development, higher activity of ROS in peduncular segment may be due to the differential seed developmental stage, which slightly



(Data represent the mean values±SE of two varieties from two seasons using three replicates;
Values denoted by different letters are significantly different at pd”0.05 following Duncan's multiple range test);
Where, (A) SOD; (B) CAT and (C) POX
Fig 8. Effects of position of ovule in different fruit segments on antioxidant enzymes from seeds across cucumber varieties, seasons and environments

lagged behind as compared to seeds from middle and/or stylar segment. Whereas, increased activity of antioxidant enzymes in peduncular segment may be due to higher rate of scavenging of these ROS. Similarly, reports with higher activity of both ROS and antioxidants were observed in immature seed of pumpkin, which reduced with the progression of seed maturity (Silva et al. 2017). Increase in antioxidants under heavy metal stress was reported by Venkatachalam et al. (2017) in *Leucaena leucocephala*. Freshly harvested seeds, irrespective of varieties, seasons and environments failed to germinate, but were viable (from TZ test; Table 1), which clearly demonstrated the presence of dormancy in seeds from all three segments of fruit. The presence of seed dormancy harvested from peduncular segment was stronger than stylar and/or middle segment. There was varietal difference in seed dormancy; significantly higher in Pusa Uday, indicated that seed dormancy was genotypic trait and not influenced by the environment. This might be due to physiological (ABA content) or morphological factors (presence of proanthocynadines in seed coat), which are under the regulation of ROS and antioxidants (Aroonrungsikul et al. 1997; Bailly et al. 2019; Patil 2018). The greater dormancy from the seeds of proximal than those from the distal position in *Isatis violascens* (*Brassicaceae*) raceme was reported (Lu et al. 2017); so also seed mass was greater in distal than those to proximal parts of the inflorescence (Mazer and Dawson 2001). Similarly, presence of primary dormancy was reported in developing cucumber (Jing et al. 2000), which developed in two phases: first one occurred during early- and mid-development (shallow dormancy), whereas second occurred in later maturation phases (deep dormancy). Therefore, two ABA peaks were observed (20 & 45 DFP) in cucumber (Aroonrungsikul et al.1997; Ahmad 2018). Application of GA or pre-chilling could overcome the seed dormancy and induce germination (Patil 2018).

Conclusion

It may be inferred from the present study that the seeds from stylar and/or middle fruit segment had temporal and spatial advantages in receiving the vigorous pollen and garnering resources than those seeds from peduncular segment. Further, morphological, physiological and biochemical assays showed that the seeds obtained from middle and stylar segments of fruit were superior in quality and yield than peduncular segment. Hence, it is recommended that optimum quality seeds in cucumber be harvested from middle and/or stylar segments; being seeds closer to style have the higher probability of seed setting than peduncular end.

सारांश

खीरा की 2 किस्मों (पूसा बरखा एवं पूसा उदय) मध्यम और स्टाइलर फल खण्डों से प्राप्त बीजों ने मुक्त प्रक्षेत्र (ई.–1) और संरक्षित (ई.–2) दोनों वातावरणों के दौरान बीज उपज गुणवत्ता में बेहतर प्रदर्शन भा.कृ.अनु.प.–भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली में किया। इसके अलावा संरक्षित वातावरण (ई.–2) से प्राप्त बीज मुक्त प्रक्षेत्र से प्राप्त बीज की तुलना में बेहतर थे। यह स्टाइलर खण्ड के अस्थाई व स्थानीक लाभ के कारण ओजपूर्ण गुणवत्ता वाले परागकण से परागण तथा अधिक मात्रा में खाद्य पदार्थ (शर्करा, प्रोटीन, स्टार्च एवं वसा) मिलने के कारण अधिक फल बन सकता है। फलों के मध्यम एवं स्वाइलर खण्ड से प्राप्त बीज के अधिक डिहाइड्रोजिनस एंजाइम, एण्टीआक्सीडेन्ट एंजाइम (एसओडी, केटेलेज एवं परआक्सीडेस) एवं कम आरओएस क्रियाशीलता (हाईड्रोजनपेराक्साइड एवं आक्सीजन), कम विद्युत चालन, कुल शर्करा एवं कुल प्रोटीन (बीज विक्षलान) बीज की बेहतर भौतिक एवं शारीरिक अवस्था का संकेत देते हैं। पेल्कुलर खण्ड से प्राप्त पिछड़े हुए बीज विकास व पकाव के कारण अधिक समय तक सुसुप्तावस्था होता है। इसलिए खीरे के इष्टतम गुणवत्ता व अधिक उपज प्राप्त करने के लिए मध्यम एवं स्टाइलर भाग से बीज लेना चाहिये।

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