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Influence of fruit load per vine on seed quality in cucumber (*Cucumis sativus* L) grown under open field and protected environments

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ABSTRACT

One fruit/vine (T1) recorded higher seed quality (germination and vigour) with compromised seed yield (assimilate transfer, the total number of sees/fruit, total number of filled seeds/fruit, seed yield/plant and 1000-seed weight) than those to two fruits/vine (T2), whereas, the three fruits/vine (T3) recorded significantly lower both seed quality and yield in two cucumber varieties (Pusa Barkha and Pusa Uday) under both open field (E1) and protected (E2) environments, during 2019, at ICAR-IARI, New Delhi. Total soluble sugars, total soluble proteins, starch and oil contents in seeds were maximum with T1. The ROS (O2·-and H2O2) and antioxidant enzymes (SOD, CAT and POX) were increased with the increased fruit load/vine, indicating the seed's physical and physiological status. Seed dormancy was not influenced by the number of fruit loads but by its genotype. Therefore, the retention of two fruits per vine was recommended to get better seed yield with optimum seed quality.

Keywords: *Cucumis sativus* L, Fruit load/vine, Seed quality, ROS, Antioxidants

INTRODUCTION

Seed production in cucumber has a serious problem of formation of higher number of under developed seeds with reduced quality. Consequently, in India minimum germination standard for cucumber is only 60%, which is very low to meet any international seed trade (90%). One of the possible solutions of this problem will be the plant load management through fruit regulation. Various workers have reported the influence of number of fruits per plant on seed yield and quality in bitter gourd and pumpkin (Huang *et al.*, 17; Kumar *et al.*, 19). However, allowing more number of fruits per plant may give higher yield with poor seed quality and vice-versa. Higher number of fruits per plant affects the assimilate transfer, that results in higher unfilled seed. Hence, the regulation of fruit loads through fruit retention per vine need to be investigated.

The number of fruits per plant along with number of seeds per fruit and individual seed weight directly decide the seed yield and indirectly influence the seed quality in cucumber (Nerson, 22). In cucurbits, among the several female flowers, only a small number of initial flowers develop into mature fruits (Diggle, 13). Baniel *et al.* (7) demonstrated that the cucurbit plants itself have an inbuilt mechanism to regulate the fruit load, in which developing fruits prevent the younger ovaries from setting. Such pollinated ovaries remain in a reversible ‘stand-by’ state until the older fruits are removed. During cucurbit seed production, the fruits are allowed to remain on the plant until seed maturation, which require more amount of food accumulates than normal fruit development. Besides, most of the flowering plants including cucurbits produce more ovules than the number of seeds (Stephenson, 25). The conversion of these ovaries into fruits and ovules into mature seeds may be limited either by the poor quality and quantity of pollens or resource limitations (nutrients and/or photosynthates) or biotic (pest and diseases) and abiotic stresses (Diggle, 13).

Jing *et al*. (18) showed the presence of primary dormancy in developing seed of cucumber, where the role of intrinsic ABA and/or some germination inhibitors were expected (Aroonrungsikul *et al.*, 5; Patil, 23). The fate of the seed, whether it will germinate or remain dormant, is regulated by ABA/GA; and this ratio is regulated by ROS under the strict supervision of antioxidants (Bailly, 6). Although, Nerson (22) and Jing *et al.* (18) documented the effects of fruit retention on various seed and fruit parameters, but until date no study has been targeted for seed composition, antioxidants and ROS activities on seed quality & yield versus fruit load/vine. Therefore, the present study was aimed to study the physiological and biochemical changes associated with fruit regulation and standardization of fruit load per vine, under open field and protected

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environments, to obtain better seed quality and higher yields.

MATERIALS AND METHODS

Present study was conducted with two cucumber varieties *viz*, Pusa Barkha and Pusa Uday (procured from Division of Vegetable Science, ICAR– Indian Agricultural Research Institute, New Delhi (IARI)), 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. The crop was planted on raised beds under two seasons (*viz,* summer-2019 & *kharif*-2019 under E1, and summer-2019 & winter-2019 under E2 environments). Five to ten flowers, from each vine were hand pollinated (7-10 am) and tagged. Further, one (T1), two (T2) and/or three (T3) fruits/vine were retained. The fruits were harvested on 45-days from pollination (DFP) under E1, whereas on 60 DFP under E2. Seed quality parameters *viz,* seed germination percentage and moisture content were measured following ISTA rules (Anon, 3), whereas vigour indices as per Abdul-baki and Anderson (1). The seed yield index [seed yield (g)/fruit weight (kg)] was calculated for each treatment (Nerson, 22).

The electrical conductance (EC) was measured and expressed as $\mu S\ cm^{-1}g^{-1}$. Total soluble sugars (TSS) and proteins (TSP) from seed leachates with different treatments were measured following Dubois *et al.* (14) and Bradford (9), respectively. Starch content in seeds was estimated using Anthrone method (Hodge and Hofreiter, 16). Seed oil content was determined using a Soxhlet apparatus (AOAC, 4). Superoxide anion ($O_2^{\cdot-}$) was measured (NBT reduction) and expressed as $\Delta A_{540}\ min^{-1}g^{-1}{}_{FW}$ following Chaithanya and Naithani, (10). H_2O_2 was estimated (formation of titanium-hydro peroxide) and expressed as µmol $H_2O_2\ g^{-1}{}_{fw}$ (Mukherjee and Choudhari, 21). SOD was assayed (inhibition of photochemical reduction of NBT) following Dhindsa *et al.* (12). Activity of catalase was measured (quantifying the residual H_2O_2 in the reaction mixture) and expressed as $\mu mol g^{-1}{}_{fw} min^{-1}$ (Aebi *et al.*, 2). POX was assayed (formation of tetraguaiacol ($\in$= 26.6mM^{-1}cm^{-1}) and expressed as µmol tetra-guaiacol (min^{-1}g^{-1} fw) formed (Rao *et al.*, 24). The experiment was conducted following CRBD in three replicates, in a bifactorial scheme and subjected to ANOVA. The germination percentage were transformed as arc sine values before statistical analysis. Statistical analyses were carried out using WASP 2.0 and Microsoft Excel 2019.

RESULTS AND DISCUSSION

The formation of higher number of under developed seed in cucumber is a serious problem; may be due to improper pollination or lack of plant energy to fill all the pollinated seeds. This could be managed through pollination or plant energy management, respectively. The plant energy could be managed by fruit load regulation or by exogenous application of micronutrients/hormones. In the present study, pollination was ensured by hand pollination and addressing the fruit load (retention of chosen number of fruits to mature on the mother plant). Allowing more number of fruits on mother plant may result in higher seed setting with poor seed quality, whereas less number of fruits improved the quality of seed with compromised seed yield. Cucumber plants produce sufficient number of female flowers and more than required male flowers on a vine in a growing season. But, pollination of all female flowers (ovaries) does not allow it to convert into mature fruits. The cucurbits plants itself have an inbuilt mechanism of fruit set inhibition (regulation of fruit load/plant). Presence of older developing fruits prevents the younger ovaries from seed setting (Baniel *et al.*, 7). The photosynthetic activities of developing fruits and seeds do not produce enough resource to support their own development. The first fruit formed on the basal node of a vine has larger share in photosynthates than the subsequent fruit formed on the upper node; thus, the first formed fruits are bigger and have suppressing effect on subsequent fruits.

Irrespective of varieties and seasons, significant differences were observed for fruit load on various fruit and seed parameters between two growing environments. The T1 (*ie,* retaining one fruit per vine), showed higher mean values of fruit characters *viz* weight (434.0 and 541.3g), length (23.15 and 25.15cm), width (6.48 and 7.41cm), cavity width (4.50 and 5.01cm) and flesh thickness (1.54 and 1.76cm) under E1 and E2, respectively. These parameters were significantly different with T2 (*ie,* retaining two fruit per vine), except for fruit length (21.50 and 23.83cm) and flesh thickness (1.44 and 1.63cm), and with T3 (*ie,* retaining three fruit/vine), except flesh thickness (1.39 and 1.53 cm) than those to T1 (Fig. 1). These results may be ascribed as the smaller and targeted active sink in case of T1 facilitated accumulation of all photosynthates without having any competition, whereas with increase in number of fruits per vine (sink) increased competition for food reserves; the size and capacity of source (number of leaves) remain consistent.

The T1 also showed significantly higher mean values of seed characters *viz,* total number of seeds/fruit (445 and 464), number of filled seeds/fruit (357 and 371.25), percent filled seed (80.74 and 80.02), number of unfilled seed/fruit (88 and 92.75), percent

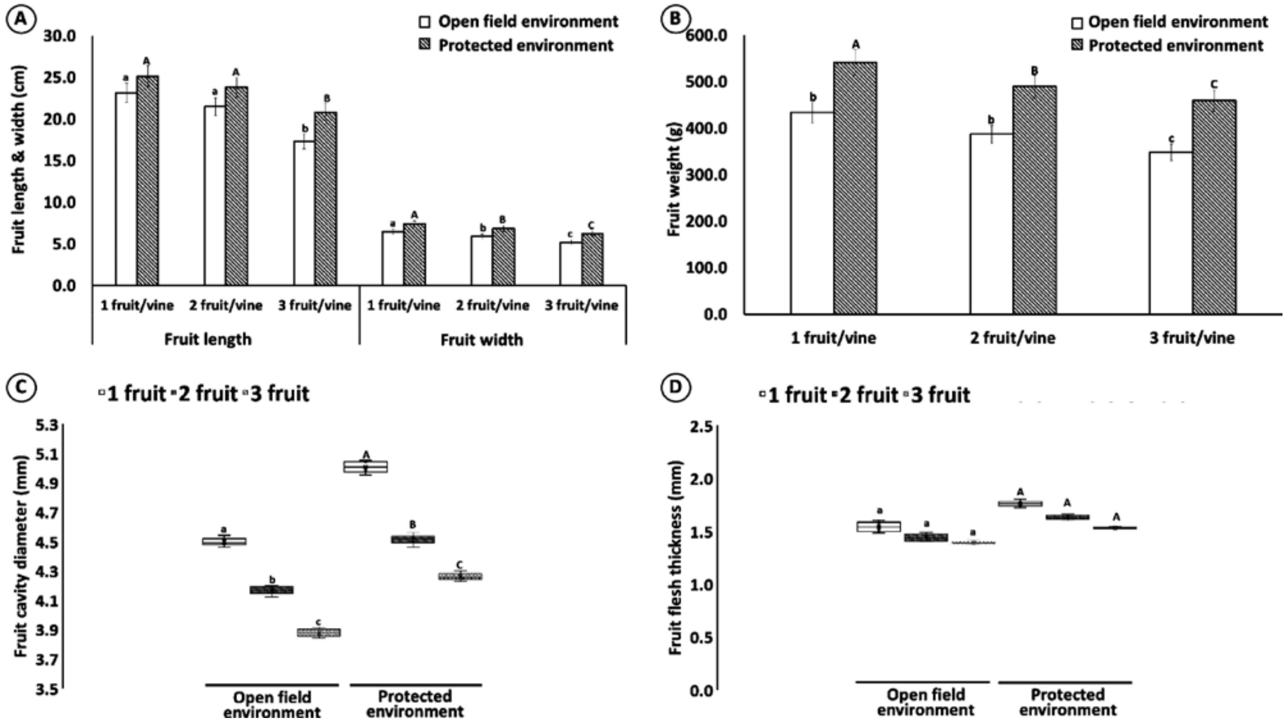

Fig. 1. Effects of number of fruits/vine retained on fruit traits across cucumber varieties, seasons and environments; where (A) fruit dimensions, (B) fruit weight, (C) fruit cavity diameter and (D) Fruit flesh thickness.

(Data are mean value±SE of two varieties from two seasons using three replicates; Values denoted by different letters are significantly different at p≤0.05 following Duncan's multiple range test)

unfilled seed (19.76 and 19.98) under both E1 and E2, respectively. Seed breadth, irrespective of varieties, seasons and environments, was consistent, whereas, the mean seed length was maximal with T1 (11 & 11.34mm) and minimum with T3 (9.86 & 10.10mm) under E1 and E2 environments, respectively (Fig. 2).

Irrespective of varieties and seasons, treatment T1 also showed significantly higher mean values for 1000-seed weight (25.14 and 27.13g) and seed yield index (20.7 and 18.6) under E1 and E2 environments, respectively (Fig. 2). The higher values of above seed yield traits could be attributed to better fruit developmental traits in bigger fruits. The negative correlation of fruit size with number of fruits/vine retained, and the positive influence of fruit size on seed size and yield have been reported in pumpkin (Devdas *et al.*, 11) and ash gourd (Mini *et al.*, 20). However, the maximal seed yield/plant was observed with T2 (*ie,* retention of two fruits/vine) (12.05 and 13.61g), thereby, significantly maximal seed yield/ha (264.1 and 298.1kg) under E1 and E2, respectively (Fig. 2). More and targeted accumulation of food reserves in larger fruit from T1 followed by T2 could be attributed to differences in seed yield parameters.

Seed composition showed the significant decrease in TSP, TSS, total starch and oil contents with increased fruit load. TSP, irrespective of varieties and seasons, were 1.14-folds & 1.43-folds higher under E1, whereas it was 1.10-folds & 1.29-folds higher under E2 with T1 than T2 and/or T3, respectively. TSS, total starch and oil contents, irrespective of varieties, seasons and environments, were 1.16-folds & 1.32-folds higher; 1.11-folds & 1.19-folds higher; and 1.03-folds & 1.15-folds higher with treatment T1 than T2 and T3, respectively (Fig. 3).

The seed quality traits, based on mean values (with two varieties under two seasons), showed that treatment T1 registered higher germination (79.00 and 84.25%) but at par with seeds from T2 (75.36 and 80.09%); whereas, treatment T3 recoded significant lower values (58.31 and 61.77%) under both E1 and E2 environments, respectively. Likewise, superior performance with treatment T1, were recorded for seed vigour index-I (1457.6 & 1836.7) at par with T2, (1292.9 & 1611.7); and seed vigour index-II (12.86 & 14.28) at par with T2 (10.98 & 12.21) under both E1 and E2, respectively (Fig. 4). T3 showed minimal seed vigour. Irrespective of varieties, seasons and environments, differences were non-significant for moisture content, whereas significant for EC.

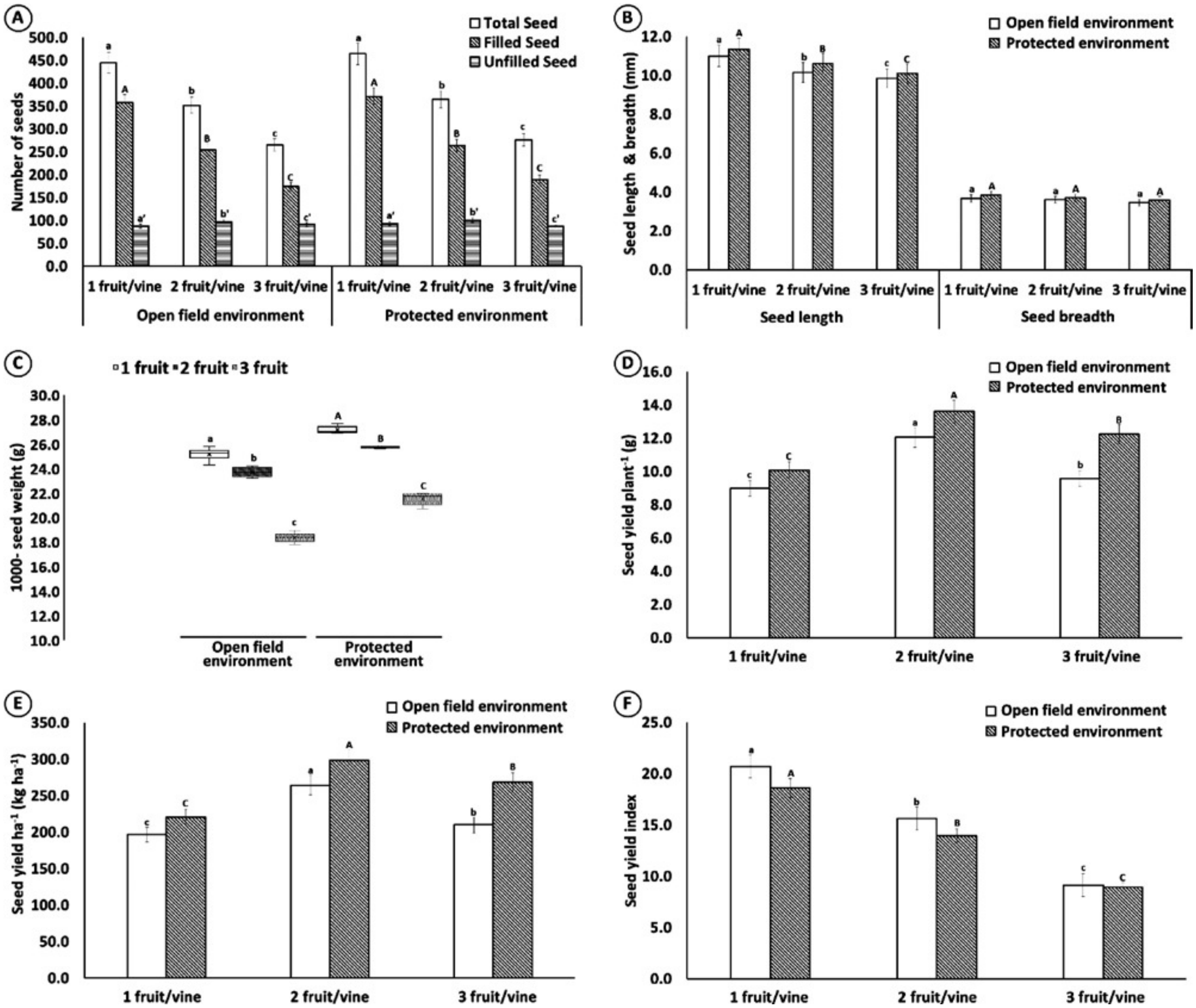

Fig. 2. Effects of number of fruits/vine retained on seed yield traits across cucumber varieties, seasons and environments; where (A) number of seeds/fruit, (B) seed dimensions, (C) 1000-seed weight, (D) seed yield/plant, (E) seed yield/hactear and (F) seed yield index.

(Data are mean value±SE of two varieties from two seasons using three replicates; Values denoted by different letters are significantly different at p≤0.05 following Duncan's multiple range test)

Seeds from treatment T1 recorded lower values of electrical conductivity (98.0μS $cm^{-1}g^{-1}$) than those seeds extracted from T2 and/or T3 (Fig. 4). The differences in seed quality performance could be due to differential accumulation of assimilates, which is directly correlated with seed size and weight; whereas, the differences in seed vigour could be attributed to differences in EC from seed leachates. Large seed size implies vigourous seedling, giving the plant an early start in light of interception and assimilation. These results were in confirmity with the performance in watermelon (Bellad and Hiremath, 8), pumpkin (Kumar *et al.*, 19) and cucumber (Jing *et al.*, 18).

Irrespective of seasons, environments and treatments, freshly extracted seeds from cucumber fail to germinate, exhibits dormancy, which was significantly higher in Pusa Uday than Pusa Barkha. Results showed higher seed viability in Pusa Uday (69.33-82.25%) than Pusa Barkha (71.75-84.33%). Seed were germinated either with GA (0.02%) treatments (Patil, 23) or when tested on 45-days from storage. Seed dormancy was not influenced by the fruit load but influenced only by genotypic differences (Table 1). Similar observations were reported in ash gourd (Garnar *et al.*, 15).

Irrespective of varieties and seasons, both the ROS ($O_2^{\cdot-}$ and H_2O_2) and the antioxidant enzymes

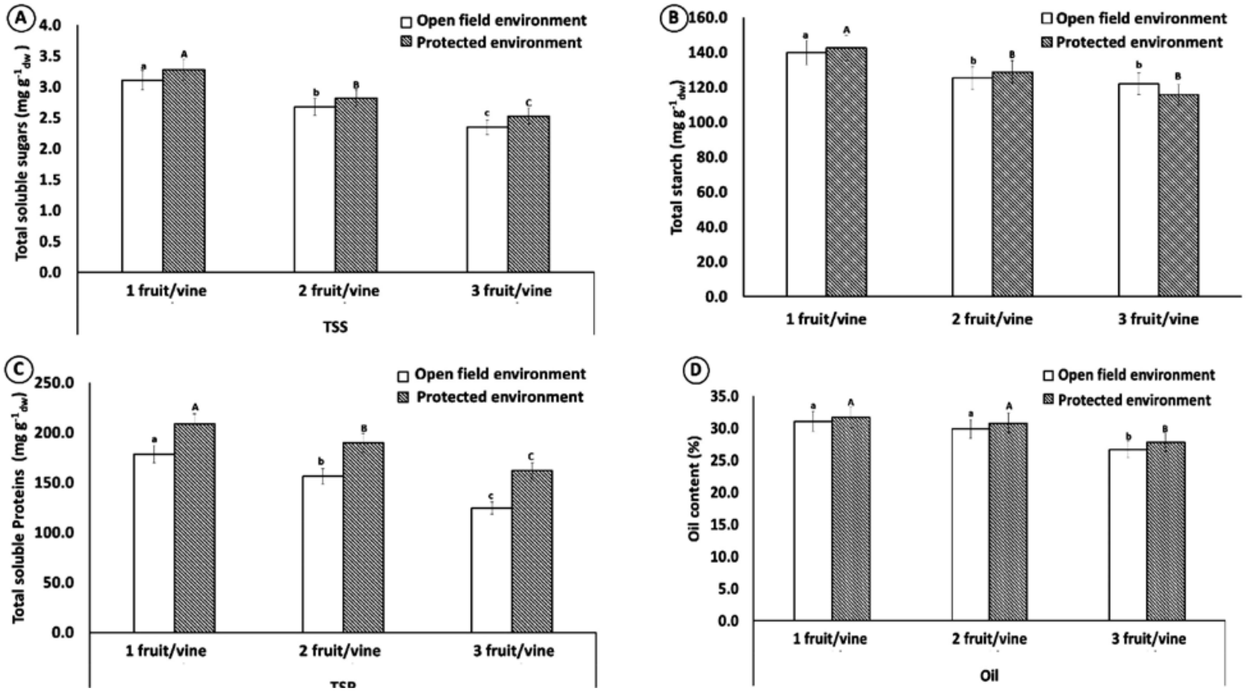

Fig. 3. Effects of number of fruits/vine retained on seed composition across cucumber varieties, seasons and environments; where (A) total soluble sugars, (B) total starch, (C) total soluble proteins and (D) oil content.

(Data are mean value±SE of two varieties from two seasons using three replicates; Values denoted by different letters are significantly different at p≤0.05 following Duncan's multiple range test)

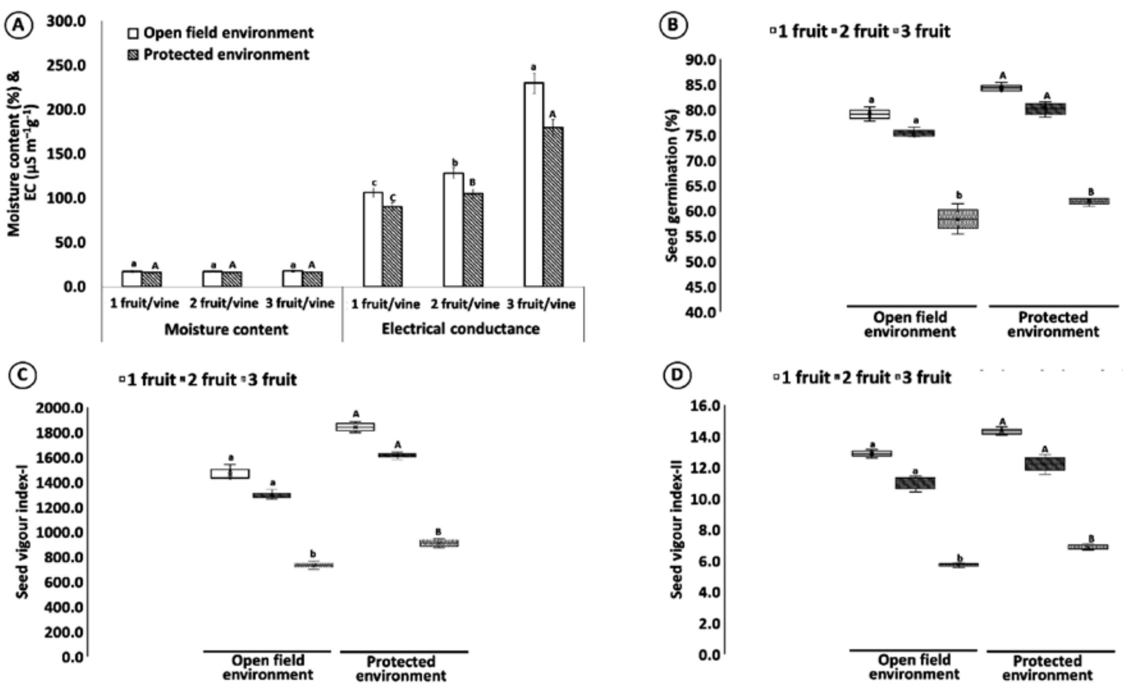

Fig. 4. Effects of number of fruits/vine retained on seed quality parameters across cucumber varieties, seasons and environments; where (A) seed moisture content and electrical conductivity, (B) seed germination, (C) seed vigour index-I and (D) seed vigour index-II.

(Data are mean value±SE of two varieties from two seasons using three replicates; Values denoted by different letters are significantly different at p≤0.05 following Duncan's multiple range test)

(SOD, CAT and POX) were significantly increased with increase in fruit load. Both H_2O_2 (1.05-folds & 1.12-folds) and $O_2^{\cdot-}$ (1.05-folds & 1.08-folds) were increased with T2 & T3 than T1, respectively (Fig 5). The antioxidant enzymes namely, CAT and POX were significantly increased with the increase in fruit load; changes in the SOD activity was non-significant (Fig. 5), indicated the physical and physiological soundness of seeds with lower fruit load. Lower ROS may also be attributed to better physical and physiological soundness of seeds; resulted in better seed vigour and storability.

Bailly (6) reported that ROS are under the control of antioxidants, decides the fate of seed *viz* germinable or remain dormant. In present study, no correlation between seed dormancy & ROS and/or

Table 1. Effects of number of fruits/vine retained on seed viability and germination across cucumber varieties.

Number of fruits/vine	Variety							
	Pusa Barkha				Pusa Uday			
	Viability from fresh seeds (%)	Germination from fresh seeds (%)	Germination from 45-days stored seeds (%)	Germination with GA (0.02%) treated seeds (%)	Viability from fresh seeds (%)	Germination from fresh seeds (%)	Germination from 45-days stored seeds (%)	Germination with GA (0.02%) treated seeds (%)
One	84.33	13.00	78.33	80.50	82.25	2.00	76.00	79.75
Two	82.50	9.50	71.00	75.61	80.43	0.00	68.33	74.33
Three	71.75	8.00	56.50	58.44	69.33	0.00	52.00	56.50
CD @ 5%	2.17	-	3.74	4.12	1.99	-	4.05	4.27
CV%	4.34	-	5.15	6.16	5.38	-	7.05	5.49

Note: Values given were arc sin transformed prior to statistical analysis

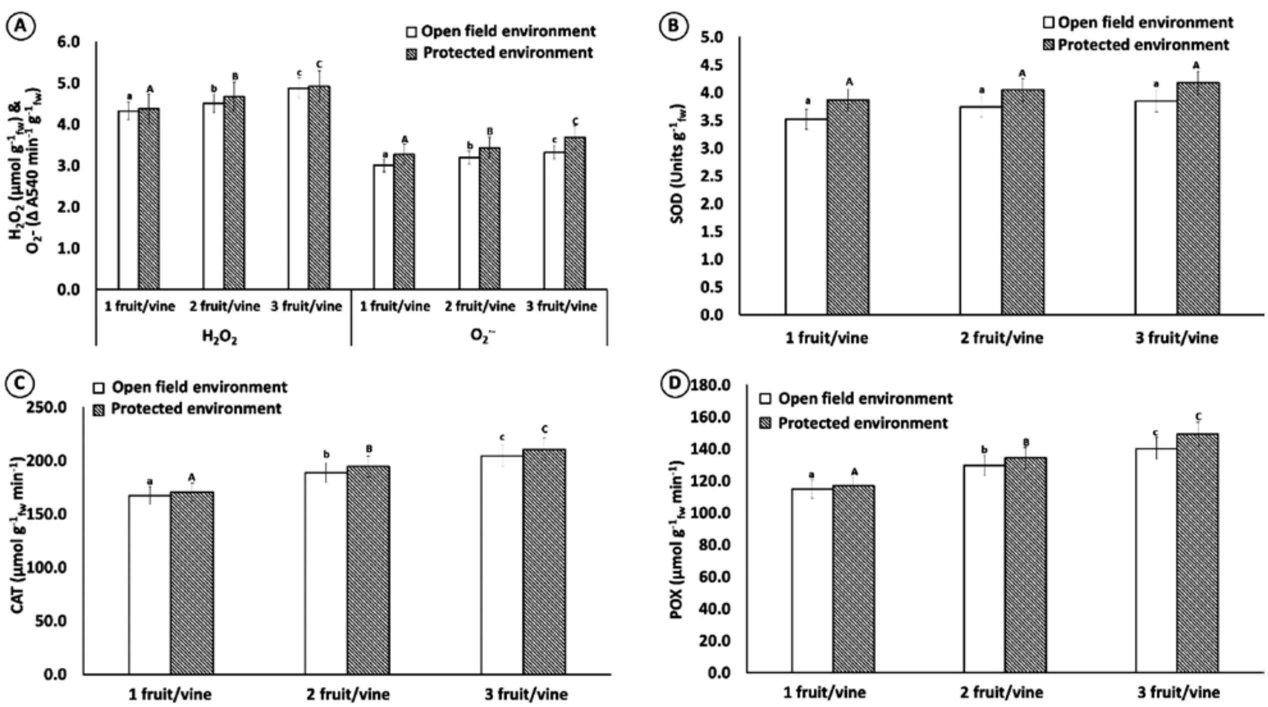

Fig. 5. Effects of number of fruits/vine retained on ROS and antioxidant enzymes in cucumber seed across varieties, seasons and environments; where (A) ROS (H_2O_2 and $O_2^{\cdot-}$) activity, (B) SOD activity, (C) CAT activity and (D) POX activity.

(Data are mean value±SE of two varieties from two seasons using three replicates; Values denoted by different letters are significantly different at p≤0.05 following Duncan's multiple range test) antioxidant activity could be established with different treatments. The differences in ROS and antioxidants were significant with different fruit loads, which could influence the seed quality *ie*, seed vigour. Further, the role of intrinsic ABA and/or some germination inhibitors were expected in regulation of dormancy (Aroonrungsikul *et al.*, 5; Patil, 23); storage of seed for a few days or treatment with 0.02% GA may change the level of these germination barriers and allowed them to germinate (Table 1).

Various morphological, physiological and biochemical changes observed in present study inferred the influence of fruit load on seed quality and yield in cucumber. Bellad and Hiremath (8) demonstrated that the fruit load could also be regulated by modulating plant population or by exogenous application of growth regulators (hormones, nano-particles, etc). Although, many fruit and seed development attributes were higher with treatment T1 *ie,* retention of one fruit per vine, but treatment T2 *ie,* retention of two fruits per vine gave higher seed yield without compromising seed quality. As the three fruits load per vine recoded significantly poor performance (seed yield and quality). Thus, from the present study, it could be concluded that retention of two fruits per vine may be recommended to get optimum seed yield and quality. The differences in the performance of seed from different fruit loads are due to the differential physiological and biochemical condition ie, TSS, TSP, starch, oil content, ROS and antioxidant enzymes.

AUTHORS' CONTRIBUTION

Conceptualization of research (NG, SKJ and AA); Designing of the experiments (NG, BST, JS and AKS); Contribution of experimental materials (BST and JS); Execution of field/lab experiments and data collection (NG and AA); Analysis of data and interpretation (NG and SKJ); Preparation of the manuscript (NG and SKJ).

DECLARATION

The authors declared no conflict of interest.

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