Morpho-physiological and biochemical changes during seed development in cucumber (*Cucumis sativus*)

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ABSTRACT

Morpho-physiological and biochemical changes in developing cucumber (*Cucumis sativus* L) seeds were monitored under both open field (E1) and protected environments (E2) during 2019 at ICAR-IARI, New Delhi. Fruits developed from hand pollinated flowers, in Pusa Barkha and Pusa Uday varieties were harvested at five-day intervals. The environment influenced both days to maturity and development of fruit and/or seed. Irrespective of varieties, seasons and environments, seed moisture decreased (over three folds); seed fresh and dry weights increased (one & half and three folds, respectively). Electrical conductance (EC), total soluble sugars (TSS) and total soluble proteins (TSP), from seed leachates, followed a bell-shape pattern. The reserve accumulation (TSS, starch, TSP and oil) registered an increase both in seed fresh and dry weights; the seed filling continued up to maturity. The ROS activity (H_2O_2 & O_2-) initially increased then decreased significantly up to maturity. A significant decrease in antioxidant enzymes indicated seed desiccation resistance. Seeds attained maximum germination (70.0 & 73.2%), seed vigour-I (1114.3 & 1323.1), seed vigour-II (8.61 & 9.55) during 40 DFP & 50 DFP; and seed dry weights (19.9 & 20.15 mg/seed) under E1 & E2, respectively.

Keywords: Antioxidants and reserve accumulation, Cucumber, Seed quality, ROS, Seed development

Cucumber (*Cucumis sativus* L, 2n=14), a member of *Cucurbitaceae*, is the most important and low-calorie vegetables consumed as salad. The peel and seeds, the most nutrient-dense part, contain phyto-nutrients, fibre & antioxidants, helps human immunity and prevent cancer. Poor seed yield and quality (formation of higher number of unfilled or under-developed seed) is a serious problem in cucumber seed production. Indian minimum seed certification standard for cucumber seed germination is 60%, very low to meet any international trade requirements (90%). The quality of seed produced depends on various factors, viz. soil, climate, cultural practices and the stage of harvest (Delouche 1980). In cucumber, the fleshy fruit, an early harvest lead to higher number of immature and/or unfilled seeds, whereas delayed harvest not only result in fruit drop, bird damage, pest and diseases but also fast ageing or vivipary. This necessitated investigating the appropriate time/stage of seed harvest to obtain better quality seeds.

Seed physiological maturity and the optimum fruit harvest time are closely related. At physiological maturity, seed filling ends, attains maximum vigour and potential germinability, which starts declining (Harrington 1972). Establishment of optimum harvest stage in cucumber is difficult, due to its indeterminate growth and extended flowering period. Seeds, keeps on developing and of various stages are present on the plant simultaneously. Physiological markers for stage of seed harvest with maximum quality are having higher seed dry matter accumulation, fruit colour, moisture content and days from anthesis, besides generation of ROS and antioxidants. The present study was undertaken to investigate the association of antioxidants and ROS during seed development, enabling identification of the optimum stage of quality seed harvest under two environments.

MATERIALS AND METHODS

Present experiment was conducted during 2019 under two environments (i) open field (E1-at Research Farm of Division of Vegetable Science),and (ii) protected (E2-at Centre for Protection Cultivation Technology (CPCT), in ICAR– Indian Agricultural Research Institute, New Delhi; with two cucumber varieties: Pusa Barkha and Pusa Uday (procured from Division of Vegetable Science); grown on raised beds under two seasons, viz. summer and *kharif* 2019 under E1 and summer and winter 2019 under E2.

5-10 flowers/vine were tagged and hand pollinated (7-10am); 2-3 fruits/vine were retained. Fruits, from these tagged flowers, were harvested (from pollination to maturity) at 5-days periodic intervals (i.e. 20-45 DFP under E1, and

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20-60 DFP under E2). The fruit dimension was measured manually, whereas seed dimension was measured using a digital Vernier Caliper. Weight of fruits and seed was measured using precision balance. Seeds were dried in shed and soaked in 500 ppm GA for 16 h prior to seed germination (fresh seeds registered high mc and dormancy). Seed quality parameters, viz. seed germination and moisture content were measured following ISTA Rules (Anon 2019), whereas seed vigour indices were measured following Abdul-baki and Anderson (1973). The EC was measured and expressed as µS/cm/g; TSS and TSP from seed leachates were measured following Dubois *et al.* (1956) and Bradford (1976), respectively. Starch was determined following Anthrone method (Hodge and Hofreiter 1962). Seed oil content was determined using a Soxhlet method, following AOAC (1990). TSS, starch and TSP were expressed as mg/g_{dw}, whereas oil content was expressed as percentage on dry weight basis.

Superoxide anions (O_2^-) were measured by the ability to reduce nitro blue-tetrazolium (NBT) (Chaithanya and Naithani 1994). Hydrogen peroxide (H_2O_2) was estimated by formation of titanium-hydro peroxide (Mukherjee and Choudhari 1983). Superoxide dismutase activity (SOD) (EC 1.15.1.1) was assayed by inhibition of photochemical reduction of NBT (Dhindsa *et al* 1981). One unit of SOD was defined as the amount of enzyme required for 50% inhibition of NBT reduction. Activity of catalase was measured by quantifying the residual H_2O_2 in the reaction mixture, as catalase quenches the H_2O_2 (Aebi *et al.* 1984). Peroxidase activity (POX) was assayed by measuring the formation of tetraguaiacol ($\square$= 26.6 mM/cm) from guaiacol (Rao *et al.* 1996). The experiment was conducted following CRBD, using three replicates, in a bifactorial scheme (6 × 2 under E1 and 9 × 2 under E2, respectively) and subjected to ANOVA. The data as percentage were transformed to arc sine values prior to statistical analysis. Statistical analyses were carried using WASP 2.0 and Microsoft Excel 2019.

RESULTS AND DISCUSSION

Morphological changes during seed development and maturation: Fruit attained edible stage on 8-10 DFP, but seeds from this stage are tiny. Seed production under E2 delayed by15-20 days. Irrespective of varieties, seasons and growing environments the dimension of fruits and seeds were closely related to the fruit maturity. The quality of the seed is not only determined by its genetic background but also by the environmental conditions of the crop during seed development. Variations in environmental factors, viz. space and time; day and night temperatures; relative humidity, soil moisture and nutrients, etc. directly affect the seed development and maturation (Baskin and Baskin 2014). Optimum environment under E2 reduced the incidence of biotic and abiotic stresses, promoted the mobilization of photosynthates towards yield and seed quality rather than defense.

Irrespective of varieties and seasons, under E1 (20–45 DFP), fruits' length, width and weight were increased

Table 1   Effects of seed developmental stages on fruit and seed traits across cucumber varieties, seasons and environments

Days from anthesis	Fruit length (cm)		Fruit width (cm)		Fruit weight (g)		Seed length (mm)		Seed breadth (mm)		Seed moisture content (%)		Seed dry weight (mg/seed)	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
20	15.6	16.4	4.65	5.01	135.3	155.3	7.45	7.24	3.02	2.95	53.50	56.21	8.07	6.91
25	16.1	17.3	4.75	5.17	152.5	190.8	7.94	7.72	3.21	3.07	41.70	44.95	11.06	9.27
30	16.5	18.5	4.94	5.37	209.3	237.3	8.19	8.28	3.33	3.27	34.16	37.74	13.98	11.63
35	17.1	19.2	5.43	5.57	272.8	286.3	8.91	9.04	3.49	3.42	24.35	29.27	17.50	14.55
40	18.1	19.8	6.21	5.81	318.5	328.8	9.80	9.32	3.61	3.55	20.80	23.22	18.74	16.79
45	18.4	20.5	6.26	6.07	355.5	379.8	10.21	9.83	3.65	3.69	17.29	20.20	19.90	18.96
50		21.4		6.49		407.8		10.25		3.77		18.15		19.70
55		22.3		7.14		428.8		10.73		3.83		16.75		20.50
60		22.5		7.22		450.8		10.88		3.89		16.17		20.16
CD@5%	0.69		0.17		24.5		0.23		0.07		4.56		1.02	

Note: Values in the table are mean of three replicates of two varieties and two seasons; where E1- open field environment, E2- Protected environment.

(15.18–20.91%, 30.85–38.74% and 154-170%), whereas under E2 (20–60 DFP): (35.28–39.13%, 42.52–45.46% and 188–192%), respectively (Table 1). Seeds' length and breadth were increased, under E1 (20–45 DFP): 36.31–37.75% and 20.06–21.45%, whereas under E2 (20–60 DFP): 48.44–53.53% and 29.53–34.78%, respectively (Table 1).

Physiological and biochemical changes during seed development and maturation

Seed moisture and seed weight: The seeds in cucumber remain embedded in fleshy fruit mass throughout development. Irrespective of varieties and seasons the seed mc from 20 DFP (53.5%), reduced gradually to (-3.09-folds) and (-3.47-folds) under E1 & E2, respectively (Table 1). Decrease of fruits and seeds mc, with an advancement of maturity, was due to desiccation of fruit and seeds, these results were in confirmation with Alan and Eser (2008) in pepper.

Contrary to seed mc, fresh and dry weights of seed increased up to (1.39 or 1.53-folds) and (2.47 or 2.92-folds) under E1&E2, respectively (Table 1). Fresh and dry weight of the seeds increased rapidly initially (until 35 and 45 DFP under E1 & E2, respectively) and continued to increase at a lower rate at later seed developmental stages. Cotyledons gained most of its storage material and attained maximum dry weight (19.90 mg/seed) until 45 DFA under E1; and (20.16 mg/seed) until 60 DFP under E2 (Table 1). At full maturity, plant completely got dried; fruit skin changed from light green to brown, and warts on skin became prominent. Increase in seed fresh and dry weights along with fruits'

dimension and seeds' (number of filled seeds/fruit and seed yield/fruit) may be attributed to continuous supply and accumulation of metabolites from mother plant to seeds up to maturity, indicated an increase in sink potential of the fruit and seed. These results were in conformity with Kalyanrao *et al.* (2014) in bottle gourd.

Electrical conductance, TSS and TSP from seed leachates: Electrical conductance from seed leakage reflects cell membrane integrity which predicts the physical condition and storability of seed. EC in 20 DFP-seeds (171.8 µS/cm/g) increased up to (1.69 or 1.91-folds) during 30 DFP under E1 or 40 DFP under E2, due to substantial increase in seed size. Further, it declined with further seed development (-1.91 or -1.83-folds) up to 45 or 60 DFP under E1 & E2, respectively (Table 2). The higher amounts of TSS and TSP during early seed development were responsible for higher EC values, which substantially decreased with seed development. TSS decreased throughout the development (-1.89 or -2.08-folds) and up to 45 or 60 DFP under E1 & E2, respectively (Table 2).

TSS, total starch, TSP and oil content in seeds: Irrespective of varieties and seasons, seed composition showed significant variations with respect to growing environments. TSS significantly reduced over (-2.50-folds) with the mean values (6.74 to 2.68 mg/g$_{dw}$) or (7.14 - 2.83 mg/g$_{dw}$) under E1 & E2, respectively (Fig 1). The data showed a strong but a negative correlation between seed development and TSS accumulation (r =-0.99, P<0.05). Total starch contents in seeds followed nearly a reverse trend of sugar accumulation, which upsurge constantly

Table 2　Effects of seed developmental stages on seed quality parameters across cucumber varieties, seasons and environments

Days from anthesis	Electrical conductance (µS/cm/g)		Total soluble sugars from seed leachates (mg/g)		Total soluble proteins from seed leachates (mg/g)		Seed germination (%)		Seed vigour index-I		Seed vigour index-I	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
20	179.1	164.5	1.727	1.864	11.18	11.64	0 (0)	0 (0)	167.7	119.6	0.00	0.00
25	240.8	177.5	1.583	1.946	13.24	11.62	17.4 (24.7)	11.79 (20.1)	312.0	222.2	0.96	0.80
30	302.7	189.5	1.575	1.746	14.03	13.18	27.2 (31.4)	19.92 (26.5)	660.3	498.8	1.70	1.73
35	224.8	280.7	1.120	1.709	11.44	12.98	49.6 (44.8)	36.09 (36.9)	1114.3	795.9	4.40	3.67
40	174.5	314.8	1.002	1.768	9.67	13.24	70.0 (56.8)	47.89 (43.7)	950.5	1028.6	8.61	5.65
45	126.2	241.9	0.913	1.234	8.88	10.62	64.4 (53.4)	61.42 (51.6)	167.7	1323.1	7.25	7.30
50		206.0		1.125		9.71		72.29 (58.2)		1261.4		9.55
55		143.7		1.000		9.13		70.09 (56.8)		1159.7		9.12
60		111.7		0.899		8.98		67.42 (55.2)		119.6		8.29
CD@5%	26.3		0.88		0.43		2.51		67.3		0.41	

Note: Values in the table are mean of three replicates of two varieties and two seasons; Values in parenthesis are arc sin transformed.

until maturity. It increased (1.64 or 2.03-folds) under E1 & E2, respectively (Fig 1). There was a linear but strong correlation between seed development and starch content accumulation (r = 0.99, P<0.05). The decrease in TSS and increase in starch contents, during seed development, may be due to the conversion of sugar into starch. Increase in starch content was also supported by Bhattacharya *et al* (2002); starch is the seed reserve, required during seed germination. Contrary to TSS, TSP in developing seed increased up to 3.49–folds. TSP in developing seed increased rapidly from 20-35 DFP (59.23–130.64 mg/g_{dw}), followed a gradual increase until 45 DFP (156.07 mg/g_{dw}) under E1,whereas it increased rapidly (54.28 - 170.62 mg/g_{dw}) during 20-50 DFP, followed a gradual increase until 60 DFP (189.57 mg/g_{dw}); indicated a strong and +ve proximity (r= 0.98, P<0.05) between TSP and seed development (Fig 1). Alike TSP, Oil, in cucumber seed is another important constituent, not only used as edible but also as pharmaceutical, cosmetic and industrial product; increased up to 3.33-folds. It increased rapidly (11.45–24.65%) under E1 (20-35 DFP), followed a gradual increase until (29.87%) during 45 DFP; also increased rapidly (9.23–28.59%) under E2 (20–50 DFP), followed by a gradual increase (30.77%) until 60 DFP. It also indicated a strong and +ve proximity (r= 0.99, P<0.05) between seed oil and a developing seed (Fig 1). The higher seed oil was positively correlated with higher linoleic and oleic fatty acids, whereas negatively correlated with moisture content (Ngure *et al.* 2015).

All seed quality parameters were lower in early harvested fruits due to presence of a greater number of immature and/or unfilled seeds with less food assimilate. Pattern of reserve accumulation in developing seed clearly support the increase in seed fresh and dry weights during seed maturation, and further demonstrated that seed filling is continued up to later maturity stages. Similar work were reported (Silva *et al.* 2017) in hybrid pumpkin. Decrease in seed water content, followed by an increase in reserve accumulates could contribute to gain in the seed germinability; the reserve proteins supply amino acids for new proteins, and starch converts into sugar during germination.

Reactive oxygen species and antioxidant enzymes: ROS under the control of antioxidant enzymes, regulate the seed development, maturation, and dormancy release by triggering cellular events (hormone signaling) associated with germination and direct oxidation of a subset of biomolecules (Bailly 2019). The balance between ROS and antioxidants decides the fate of seed, *viz* germination, dormancy or oxidative stress caused cell death. Irrespective of varieties and seasons, the mean data on ROS activity (H_2O_2 and $O_2^{\cdot-}$), showed an initial increase followed by a significant decrease until maturity. Although the overall decrease under E1 in H_2O_2 generation was non-significant, whereas it decreased (-1.22-folds: 5.72 to 4.66 µmol/g_{fw}) under E2 (Fig 2). The activity of $O_2^{\cdot-}$ under E1, decreased (-1.19 folds: 3.82 to 3.12 Δ A540/min/g_{fw}); whereas under E2 it decreased (-1.25-folds: 4.29 to 3.42 Δ A540/min/

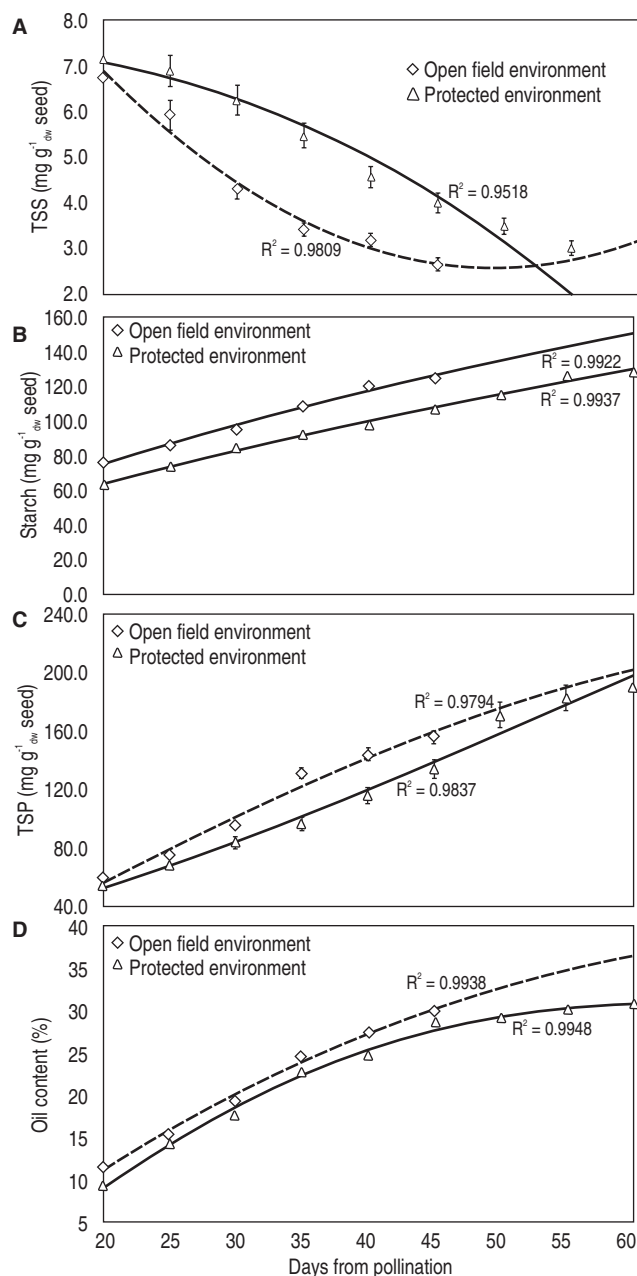Fig 1 Seed composition in developing seed across cucumber varieties, seasons and environments. (Data represent the mean values±SE of two varieties and two seasons using three replicates; Trendlines were developed from regression analysis)

g_{fw}) (Fig 2). The higher ROS activity during early seed development, where the seeds were immature having higher mc are considered as potential threat to seed quality (seed vigour). These results were in agreement with Silva *et al.* (2017) in pumpkin. SOD is considered as the first line of defense against ROS, generated through oxidative stress, dismutase superoxide anion ($O_2^{\cdot-}$) into H_2O_2 and oxygen; further H_2O_2 is detoxified by CAT and POX (Gill and Tuteja 2010). The regression analysis showed that SOD, CAT and POX activity were higher during 20 DFP

that declined significantly with seed development. The antioxidants were declined for SOD (-1.69 & -1.75-folds with r = -0.99, P< 0.05); CAT (-1.52 & -1.51-folds with r = -0.99, P<0.05); and POX (-1.81 & -2.00-folds with r = -0.98, P<0.05) under E1 and E2, respectively (Fig 2). A significant decrease in antioxidant enzymes with an advancement of seed development and maturation indicated resistance to seed desiccation. The higher activities of SOD, CAT and POX, during an early seed development, could be attributed to higher ROS activity with available more seed water in immature seed, whereas with seed maturity the seed mc declined, considered as stress factor, resulting in reduced ROS activity; further reduced antioxidant's activity showed the tolerance to desiccation. These results were in line of Silva *et al.* (2017) in hybrid pumpkin. As, the cucumber seed contains about 30% oil, therefore the role of antioxidants, especially CAT becomes more important, removes H_2O_2 generated during β-oxidation of fatty acids (Bewley and Black 1994).

Seed quality attributes: It is established that harvesting of seed at optimum time and stage is most important to obtain quality seed; maximum seed dry weight and quality was obtained during physiological maturity (Harrington 1972). This hypothesis was supported by many researchers for a long time (Alans and Eser 2008), but later studies (Siddique and Wright 2003) in many crops showed its contradiction with harvest maturity. The present findings showed that the maximum seed quality (germination and vigour) during physiological maturity, whereas there was a steady increase in seed dry weight, under E1 & E2, respectively. Irrespective of varieties and seasons, seeds attain germinability on 25 DFP; maximum mean value germination 70–73.58%; maximum seed vigour index-I (1114 and 1322.5) and seed vigour index-II (8.61 and 9.68) under E1 and E2, respectively (Table 2). Results depicted that seed attained physiological maturity with maximum seed germination and vigour on 40 DFP or 50 DFP under E1 or E2, respectively. The harvest maturity, based on seed dry weight was observed on 45 DFP (0.0114 g/seed) & on 60 DFP (0.0121 g/seed) under E1 &E2, respectively.

In cucumber, having indeterminate growth habit, practically difficult to trace all the fruits based on DFP at harvest maturity; the fruit colour and complete plant wilt stage could be the better markers for identification of harvest maturity in quality seed production. Change in fruit skin (green to yellowish-brown or brown) due to degradation of chlorophyll with prominent warts or nets and rapid loss of moisture content at maturity may give the better idea of harvesting stage. Besides, the magnitude of seed chlorophyll fluorescence may be use as an indicator for harvest of cucumber seeds (Jing *et al.* 2000).

It may be concluded that, cucumber fruits be harvested on 45 DFP under E1 and/or on 60 DFP under E2; preferably at complete plant dry stage or when fruit turns brown and warty. Further, seed crop of cucumber be preferably raised under E2 to obtain better seed quality and higher yields. The advancement of seed development and maturation,
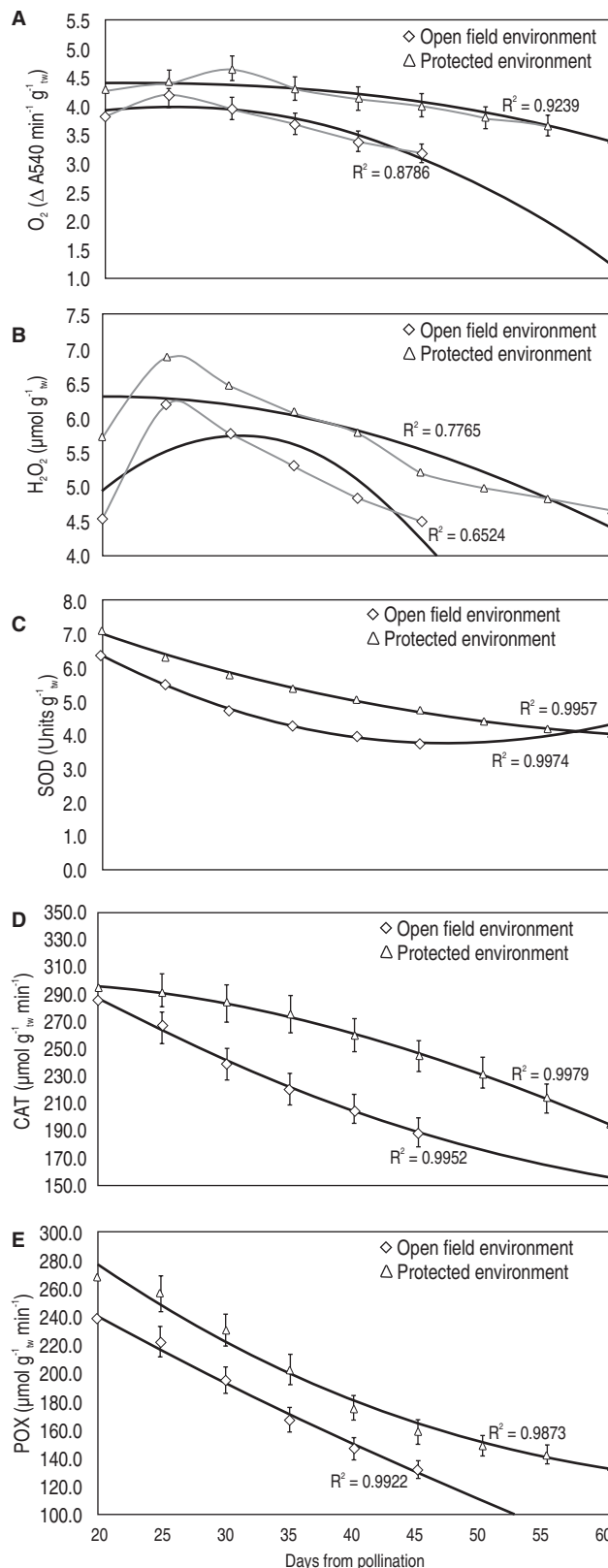

Fig 2 Activity of ROS ($O_2^{·-}$ and H_2O_2) and antioxidant enzymes (SOD, CAT and POX) in developing seed across cucumber varieties, seasons and environments. (Data represent the mean values±SE of two varieties and two seasons using three replicates; Trendlines were developed from regression analysis).

leads to reduced seed moisture, increased seed fresh and dry weights, germination and seed vigour. TSP, starch and oil contents, indicated seed filling is continuous up to last maturation stage. Immature seed with higher moisture, during early stage of seed development, causes higher threat to drying stress resulting in higher ROS activity and consequently, higher antioxidant enzymes, whereas with the advancement of maturation, the desiccation stress reduces leading to lower ROS and antioxidant enzymes. Further, a comprehensive understanding of inter conversion of photosynthates, specifically regulation of the partitioning of carbon between starch, oil and protein, acquisition of germination or dormancy during seed development and maturation needs researcher's attention.

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