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Effects of endogenous abscisic acid on fruit growth and ripening of coloured capsicum (*Capsicum annuum*)

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ABSTRACT

The present study was conducted at ICAR-IARI, New Delhi in the year 2016–17 to ascertain the role of abscisic acid (ABA) on fruit growth and ripening in capsicum. ABA was estimated at five fruit developmental stages of yellow and red coloured varieties. Irrespective of varieties higher level of ABA was recorded at 1st stage which later on showed slight reduction at 2nd and 3rd stages and thereafter, registered further sharp rise at 4th stage. Respiration rate was higher in earlier stages and later it decreased up to two fold in fifth stage. Total chlorophyll was reduced in successive developmental stages while carotenoid increased. Total carotenoid content was found to be ABA dependent and it increased with elevation in fruit's ABA content. The results indicated that ABA plays a vital role not only in fruit ripening but in early fruit growth of capsicum too.

Key words: Abscisic acid, Capsicum, Carotenoids, Fruit developmental stages, Ripening

Sweet pepper (*Capsicum annuum* L.) also known as bell pepper, belonging to family solanaceae, is an internationally important vegetable crop. It has been largely cultivated over the world for its variety of colours and rich nutritional value due to presence of vitamin A, B, C, E, flavonoids, phenolics and carotenoids (Maria *et al.* 2010). Abscisic acid, a phytohormone belongs to class of secondary metabolites known as isoprenoids. Role of abscisic acid is not only limited to abscission, dormancy, germination, growth, root geotropism, and stomatal functions (Taiz and Zeiger 2006) but it also extends to fruit ripening. It has been known that not only ethylene, ABA also plays a crucial role in the regulation of fruit ripening by increasing endogenous ABA concentration during the ripening of climacteric and non-climacteric fruits (Setha *et al.* 2004). Moreover, ABA concentration is very low in unripe fruits, but it increases during fruit ripening.

In climacteric vegetables such as melons, the level of ABA increases from maturation to harvest, while in non-climacteric vegetables like beans, the level of ABA increases before maturation and thereafter, decrease until harvest. The differing ABA levels suggest that the role of ABA may vary between vegetables and fruits (Setha *et al.* 2004, 2005). In non-climacteric fruit, the role of ABA in fruit ripening and senescence is more important than that of ethylene and ABA may trigger or start the maturation process (Yang and Feng 2015). Considering this gap (least work on ABA based ripening) and multifaceted role of ABA in ripening and fruit development, present study was carried out in capsicum. The available literature shows that no research has been carried out on regulation of ABA based fruit development and ripening in capsicum. Hence, the objective of present study is to quantify the endogenous concentration of abscisic acid and to ascertain its role in fruit development and ripening in coloured varieties of capsicum.

MATERIALS AND METHODS

The study was conducted at ICAR- Indian Agricultural Research Institute, New Delhi. Capsicum varieties. viz. red (Natasha, Inspiration), yellow (Swarna, Bachata) were grown in poly house of Centre for Protected Cultivation and Technology, IARI, New Delhi in the month of December to February, 2016–17. Fruits were harvested at five developmental stages, viz. 1st stage (21 days after anthesis (DAA)), 2nd stage (28 DAA), 3rd stage (35 DAA), 4th stage (42 DAA) and 5th stage (60 DAA), with three replicates each. Uniform and healthy fruits were brought in laboratory for measurement of fruit diameter, colour and estimation of abscisic acid, respiration rate, total chlorophyll and total carotenoid content.

Fruit diameter was measured at all five stages of fruit development by using digital Vernier calliper. Equatorial diameter was measured by keeping the fruit in between Vernier calliper horizontally while vertical or polar diameter was also taken and expressed in millimetre (mm).

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The surface color of capsicum fruit of different varieties was measured by reflectance using a colorimeter (Model CE 310; Macbeth, Tokyo, Japan). A rectangular CIE Lab system (L*, a*, and b*) was measured, and chroma (C*) value were calculated according to Kim *et al.* (2002). From each fruit, a total of three readings were recorded to avoid any miscalculation.

ABA estimation was done according to Mou *et al.* (2016) with slight modification in the procedure. ABA was extracted by grinding 1.5 g of fruit pericarp tissue using 10 ml 80% methanol (v/v) and 1 % polyvinylpyrrolidone (PVPP, w/v) in liquid nitrogen. The extracted sample was centrifuged at (10000 × g, 15 min.), supernatant liquid evaporated under reduced pressure at 35°C in rotary evaporator. The resulted precipitate was dissolved in 1.5 ml of 50 % methanol (v/v), finally filtered through 0.45 µm filter membrane and submitted to high performance liquid chromatography and was further eluted through a Sep-Pak C18 cartridge (HPLC, Waters, USA) for analysis. Samples (20 µl) was injected in HPLC for ABA estimation. The mobile phase consisted of 1 % (v/v) acetic acid (solvent A) and 100 % methanol (solvent B). It was eluted with a linear gradient of methanol (40 - 60%) at flow rate of 1 ml/min, and VWD was used as a detector in HPLC at wavelength of ABA at 260 nm. The external calibration curves were used for quantification.

Respiration rate in capsicum was measured using gas analyzer (Model: Checkmate 9900 O_2 / CO_2, PBI Dansensor, Denmark). For this, capsicum fruits were trapped in airtight one litre container. The container was kept at 35 ± 5 °C for 3 h. Major respiratory gases were accumulated at the headspace. After certain period gases in headspace were sucked by sensors fitted in gas analyser through a needle. Concentration of O_2 (%) and CO_2 (%) was recorded. Respiration rate of capsicum was measured and expressed as ml CO_2/kg/h.

Total Chlorophyll and total carotenoid content was determined by non-maceration method (Hiscox and Israelstam 1979). Fresh fruit were cut into small pieces and 50 mg were put in test tubes containing 10 ml of dimethyl sulphoxide (DMSO). The test tubes were kept in oven at 65°C for 4 h to facilitate the extraction of chlorophyll into the solution. The absorbance of solution was measured at 645 and 663 nm using double beam UV-Visible spectrophotometer (ECIL, Hyderabad, India). Total chlorophyll were calculated according to Lichtenthaler and Wellburn (1983) and expressed as mg/g FW. To calculate the carotenoid concentration, the absorbance was measured at 470 nm and expressed as mg/g FW.

Statistical analysis: The experiment was conducted in homogenous condition and data were subjected to two way ANOVA. Significant effects were noted ($P < 0.05$) and also CD was worked out for each of parameter along with standard error of mean. Data were analyzed by using SAS 9.4 software and treatment means were compared with Tukey's new multiple range test.

RESULTS AND DISCUSSION

Fruit diameter: Capsicum fruit diameter is an important parameter to determine fruit growth and its development. Fruit diameter (both equatorial/horizontal and polar/vertical) was recorded at all five stages of fruit development and it was found to increase from 1st stage (21 DAA) to 4th stage (42 DAA). Later on non significant difference was recorded between 4th and 5th stage in all the varieties (Fig 1a, b). Among varieties, Inspiration was found to have larger fruits in comparison to other three varieties. Increase in fruit diameter (vertical and horizontal) during 1st to 3rd stage may be due to cell division and cell enlargement (Watada and Morris 1967), while 4th stage growth (slower than 1st to 3rd stage) may be attributed to increase in seed number and size (Marcelis and Hofman Eijer 1995). The reason for zero growth at fifth stage could be due to cessation in cell growth of all fruit parts (Kumar *et al.* 2014). Unlike the climacteric fruits, non climacteric fruit growth depends on ABA concentration within the fruits during different developmental stages (Leng *et al.* 2014). In our findings, ABA content was found to diminish from 4th to 5th stage, which may be another reason for slower growth during last phases of fruit development.

Fruit colour: Capsicum is known for its wide range of colours like green, red, orange, yellow etc. Colour is one of

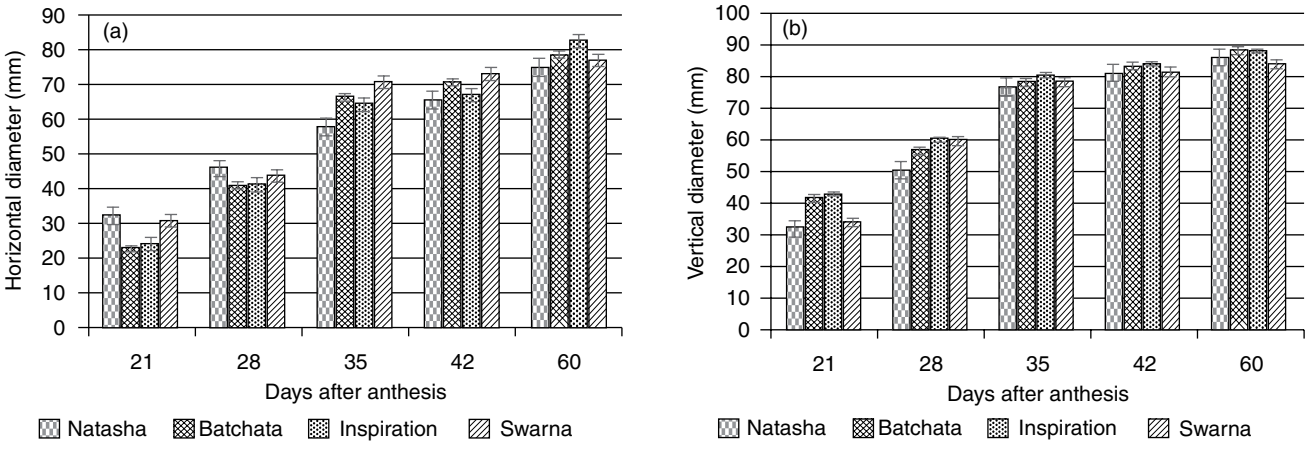

Fig 1 Fruit diameter (a) Horizontal/ equatorial, (b) Vertical/ polar, of five stage of capsicum

the most important attribute in capsicum as it is the main reason for its high demand in market. L*, a*, b* and Chroma value of Natasha, Inspiration Bachata, Swarna varied significantly. L* values indicate brightness or luminosity (0, white to 100, black); a* depicts variation from green (-) to red (+); while b* depicts variation from blue (-) to yellow (+) and chroma value indicates the saturation of colours. L* value increases from initial stage to final stage as shown in Fig 2a which indicates that while approaching towards maturity capsicum become more bright coloured as compared to immature green colour. Irrespective of colour, all the four varieties shown higher L* value at 5th stage of development. It is interesting to note that L* value was found to be elevated with increased level of carotenoid which is directly correlated with ABA concentration. Seroczynska *et al.* (2006) also found the same relation of L*, a*, b* value with carotenoids while working on winter squash. The low brightness in red coloured capsicum may be due to higher carotenoid content and variations in the visual expression of the mature stage of the fruits (Vera-Guzman *et al.* 2011). Initially up to third stage b* value was lower in all four varieties but it increased drastically in 4th and 5th stage in yellow coloured varieties (Fig 2 c). Chroma values (Fig 2 d) were calculated from a* and b* values. Fig 2c shows that b* value increases as fruit approaches its maturity and ripening stage in all the four varieties being highest in yellow variety Bachata and lowest in red variety Inspiration. b*, a* and Chroma values showed positive correlation with total carotenoid and abscisic acid content in later stages of fruit growth. The red fruits with higher a* values (Fig 2b) may be due to richer flavonoid content as found by Vera-Guzman *et al.* (2011) in pepper.

Respiration rate: There are several factors which influence fruit physiology among which respiration rate plays an important role. Respiration rate has shown a varying trend in all varieties during different ripening stages. Initially respiration rate was higher (8.90 ml CO_2 /kg/h) at 1st stage, later it reduce to half (4.07 ml CO_2 /kg/h) at 5th stage of fruit development in all four varieties of capsicum (Table 1). Among varieties Bachata had maximum respiration rate,

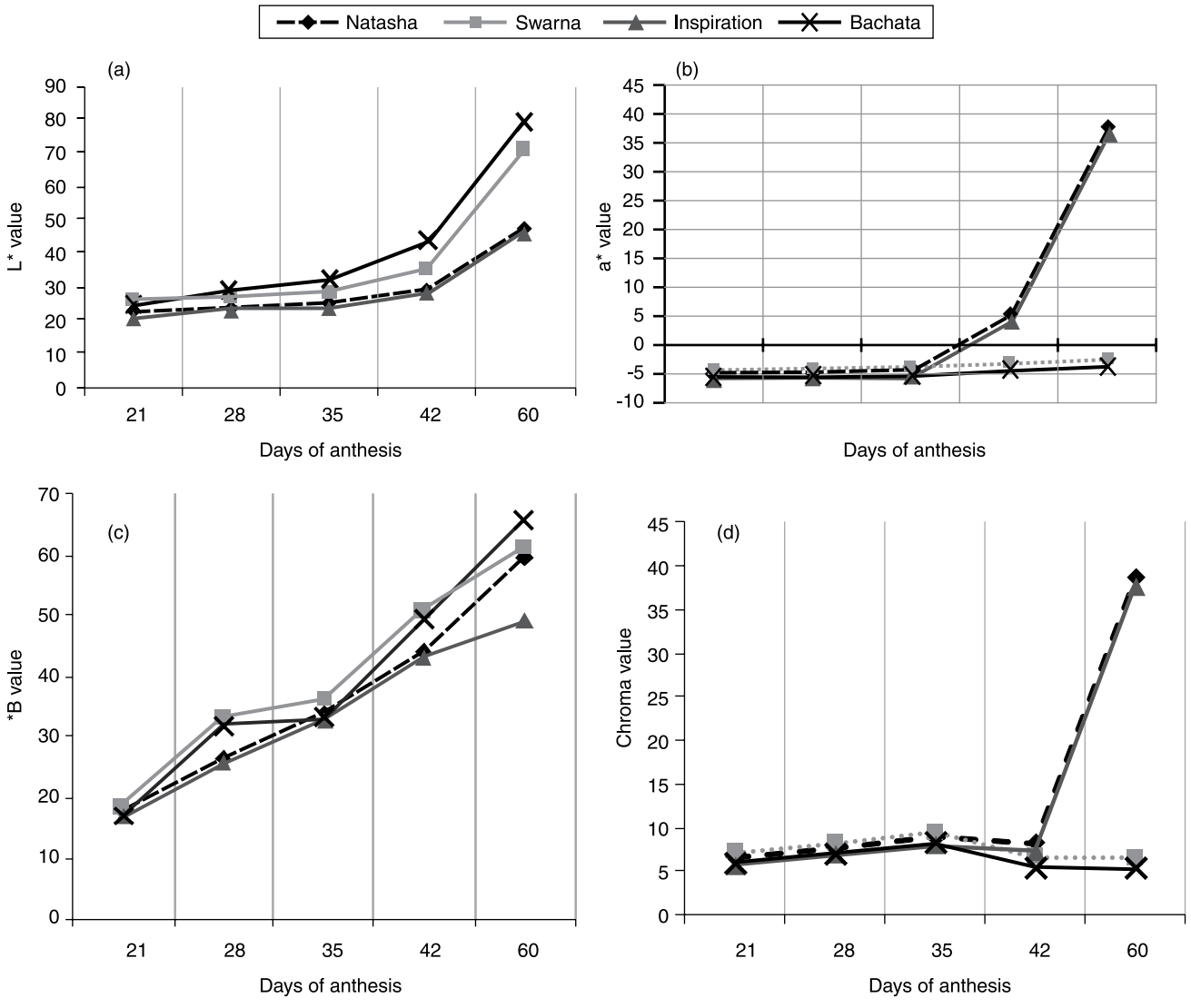

Fig 2 Fruit colour (a) L*, (b) a*, (c) b*, (d) Chroma values at five stage of capsicum

Table 1 Respiration rate and abscisic acid content at different developmental stages of capsicum

Variety	Respiration rate at various fruit developmental stages (ml CO2 /kg/h)					Mean	ABA at various fruit developmental stages (µg/g FW)					Mean
	S1	S2	S3	S4	S5		S1	S2	S3	S4	S5	
Natasha	6.28	9.20	5.86	3.03	3.75	5.62	1.21	0.93	1.40	3.95	1.71	1.84
Swarna	7.06	6.79	4.59	2.23	1.81	4.90	1.46	1.13	1.12	3.38	1.42	1.70
Inspiration	6.36	5.54	4.16	3.32	2.80	5.84	3.61	2.03	2.41	3.96	1.34	2.67
Bachata	15.88	5.67	6.35	5.27	3.91	7.42	3.35	2.41	2.54	4.01	2.18	2.90
Mean	8.90	6.80	6.49	4.21	4.07		2.41	1.62	1.87	3.83	1.66	
CD (P=0.05) for varieties				0.25	S.E.(V)	0.35				0.08	S.E. (V)	0.10
CD (P=0.05) for stages				0.32	S.E. (S)	0.39				0.10	S.E. (S)	0.12
CD (P=0.05) for varieties × stages				1.29	S.E. (V×S)	0.78				0.40	S.E. (V×S)	0.24

while minimum level was found in Swarna variety, which shows that there is no relation of yellow and red coloured capsicum with respiration rate. These results were in line with previous experiment which showed higher respiration rate in unripe stage and lower in ripened fruits in citrus (Aharoni 1967). Further, this reduced respiration rate at later stage of fruit growth may be due to increase in pericarp thickness and the deposition of waxes on fruit surface which leads to the reduction of skin permanence and low respiration rate with advancing fruit maturity (Wongmetha *et al.* 2015).

Abscisic acid content: Abscisic acid (ABA) is a stress related plant hormone known for its ability to support defence mechanism in plant and fruits under both abiotic and biotic stress condition. The result in this experiment showed that abscisic acid concentration increases during ripening process in all the varieties. There is general belief that ABA content is less during early stage and more at advance stages of fruit development. Contrary to this belief, in above findings ABA content was found to be higher in all varieties at 1st and 4th stage of fruit development. It shows that in addition to ripening, ABA plays an important role in initial fruit development also. Abscisic acid content in breaker stage (4th) was found to be maximum in variety Bachata, while lowest in Swarna. All the varieties showed significant difference in ABA content in first 4 stages while 5th stage does not show any significant difference (Table 1). The reason for higher ABA content during first stage could not be established or supported with the available research finding on this aspect. It is well established phenomena that ABA synthesis increases in fruits when they enter senescence stage during ripening (Barickman *et al.* 2014). Similar results were reported by Sun *et al.* 2012 and Wang *et al.* 2013 while working on tomato and cucumber. The variation in ABA content of different varieties may be attributed to difference in their genotype and sink-source relationship (Luo *et al.* 2014).

Total chlorophyll content: Chlorophyll content is a major factor directly related to the green colour of capsicum fruits. One of the earliest biochemical changes during fruit ripening is chlorophyll degradation. In our finding, maximum chlorophyll was found in variety Natasha and minimum in Bachata at 1st stage (21 DAA) of fruit developmental. Later at 4th stage chlorophyll content was drastically reduced in all 4 varieties. If we compare total chlorophyll content in 1st stage and 5th stage of fruit development, around seven folds reduction was observed in all the varieties (Table 2). High level of chlorophyll and ABA during first stage indicates that chlorophylls are derived from the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway which is common for both. Drastic reduction in total chlorophyll content at 4th and 5th stages may be due to degradation of chlorophyll by chlorophyllase enzyme as observed in previous studies (Barry 2010).

Total carotenoids content: Carotenoid is a very important pigment responsible for various colours present in capsicum. Unlike the chlorophyll content, total carotenoid content was found at minimum level in earlier fruit developmental stages, which later on peaked during last stage of fruit ripening. On an average, variety Natasha showed the highest level of total carotenoid content (1.64 mg/g FW) and lowest (0.56 mg/g FW) in Bachata at 5th stage of fruit development. Among different stages minimum total carotenoid was found in 2nd stage which later increases around 3 folds in Swarna variety and 5 folds in Inspiration at 5th stage of fruit development. It is interesting to note that variety and ripening stages interaction was found highly significant during stage 5th while in stage 2nd and 3rd it remained insignificant (Table 2). This increase in total carotenoid content is due to conversion of chloroplast to chromoplast which is responsible for synthesis of coloured pigments (Borovsky and Paran 2008). Another reason for the higher level of carotenoids during 4th-5th stages may be due to protection of xanthophyll cycle (de-epoxidation of violaxanthin to zeaxanthin through antheraxanthin) by ABA (Du *et al.* 2010). Previous workers also reported higher carotenoids accumulation in coloured capsicum during ripening (Ha *et al.* 2007).

The results indicated that abscisic acid plays an important role in early fruit growth (21 DAA) and regulation

Table 2 Total chlorophyll and total carotenoids at different developmental stages of capsicum

Variety	Total chlorophyll at various fruit developmental stages (mg/g FW)					Mean	Total carotenoid at various fruit developmental stages (mg/g FW)					Mean
	S1	S2	S3	S4	S5		S1	S2	S3	S4	S5	
Natasha	0.62	0.44	0.35	0.18	0.06	0.33	0.34	0.25	0.19	0.48	1.64	0.58
Swarna	0.50	0.48	0.46	0.04	0.06	0.31	0.29	0.29	0.26	0.31	0.73	0.37
Inspiration	0.45	0.27	0.33	0.18	0.07	0.26	0.24	0.14	0.15	0.50	1.35	0.47
Bachata	0.35	0.38	0.50	0.29	0.08	0.32	0.34	0.23	0.33	0.47	0.56	0.38
Mean	0.48	0.39	0.41	0.17	0.07		0.30	0.22	0.23	0.44	1.07	
CD (P=0.05) for varieties				0.010	S.E. (V)	0.014				0.012	S.E. (V)	0.017
CD (P=0.05) for stages				0.013	S.E. (S)	0.016				0.016	S.E. (S)	0.019
CD (P=0.05) for varieties × stages				0.039	S.E. (V×S)	0.032				0.064	S.E. (V×S)	0.039

of ripening. Different Capsicum varieties showed different pattern of ABA accumulation and synthesis of pigments like chlorophyll and total carotenoids. In future, work may be initiated to find out interactive role of ABA with other phytohormones and enzymes responsible for synthesis of health benefitting secondary metabolites (oleoresin, phenols, terpenes, flavonoids).

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