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Stylar end browning: a physiological disorder of Indian jujube (*Ziziphus mauritiana*) fruits

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ABSTRACT

A physiological disorder has been noted in Indian jujube or *ber* (*Ziziphus mauritiana* Lamk.) cv. Chhuhara, wherein tip (stylar end) of the *ber* fruits turned brown. The disorder has been found to aggravate with the progress in the maturity. The disorder affected fruits had less soluble solids content, reducing and total sugars and ascorbic acid; besides higher content of secondary metabolites and enzymes responsible for oxidative browning. Upon nutrient analyses, the boron content found to be higher in affected fruits in comparison to normal, healthy fruits. Since, this disorder appears during late harvesting period, when atmospheric temperature is higher, it may be concluded that high temperature and evapo-transpiration accompanied with high illumination had resulted in boron accumulation beyond its threshold level, which led to the development of browning of stylar end of fruits in *ber* cv. Chhuhara.

Key words: Antioxidant activities, Boron toxicity, Fruit maturity, Polyphenols, Stress

Indian jujube or *ber* (*Ziziphus mauritiana* Lamk.) belongs to the family Rhamnaceae and said to be an indigenous fruit crop to India. It is one of the most suitable fruit trees for arid and semi-arid regions (Krishna and Parashar 2013), where it is grown on desert soils. It is the only fruit crop which can give good returns even under rainfed conditions and can be grown in a variety of soils and climatic conditions ranging from sub-tropical to tropical (Krishna *et al.* 2014a). It is righty known as a poor man's apple. The annual production of jujube in India is estimated to be 10 lakh tonnes with the productivity of 10 metric tonnes/ha (Bal 2016). Biotic stresses like diseases and pests have been reported to cause huge economic losses in *ber* (Bal 2016), if not managed properly. However until now, physiological or abiotic stress related disorders had no significant impact on yield reduction in *ber* fruits except occasional fruit drop (Bal 2016), which is largely owed to inadequate fertilization. Recently, 'stylar end browning' disorder of *ber* fruits has been reported in cv. Chhuhara (Krishna *et al.* 2014b). Some preliminary experiments indicated that disturbed nutrient and biochemical status of fruits could be its cause. Therefore in order to ascertain the etiology of the disorder, the present study was carried out with the aim to have an insight into the cause of this disorder in Indian jujube.

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MATERIALS AND METHODS

The study was carried out in the experimental *Ber* Germplasm Block of ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan, India on 14 years old Chhuhara *ber* trees budded on *Ziziphus rotundifolia* rootstock, spaced 6 m between rows and plants. The soil of experimental site was loamy sand in texture with pH 8.23, EC 1.59 dS/m, and organic carbon 0.27%. During the experiment, a dose of 30 kg FYM and 400g N, 200g P_2O_5 and 200g K_2O/tree were applied per tree. The flowering duration in cv. Chhuhara is nearly one month long (mid September- mid October) and consequently duration of fruit set is from 3rd week of September to 3rd week of October; therefore, on a single tree fruits with variable developmental stages can be seen. For biochemical and nutrient analyses, *ber* cv. Chhuhara was harvested for their fresh fruits in the 4th week of February in 2014 and 2015. Fruits were selected according to the uniformity of shape and colour. They were picked at ripening stage and then stored at -20 ^{0}C for further studies in the laboratory. For analysis, fruits samples (250 g fresh weight/replicate) were used.

Soluble solid contents (^{0}Brix) was measured at 20ºC using an Abbe refractometor. Titratable acidity (%), ascorbic acid contents (mg/100g fresh weight; FW), total phenolics (mg GAE/100g FW), total flavonoids (qurecetin equivalents/ 100g FW), flavanols (phloroglucinol equivalents/100g FW), 0-dihydric phenol (pyrocatechol equivalents/100g FW) and antioxidant activities [cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP) and

1,1 diphenyl-2-picryl hydrazyl (DPPH)] were measured by the methods as suggested by Krishna and Parashar (2013).

Estimation of enzymes polyphenol oxidase (PPO) and peroxidase (POD) were done according to the methods suggested by Krishna *et al.* (2008) and values were expressed as unit/min/g FW and nmol of guaiacol oxidized per min per g of FW, respectively. The level of lipid peroxidation was measured in terms of malonialdehyde (MDA) by using thiobarbituric acid (TBA) reaction (Kumar *et al.* 2011).

Among nutrients, phosphorus (P) was estimated by the method of Chapman and Pratt (1961). Nutrients such as calcium (Ca) and potassium (K) were determined by flame photometer. Boron (B) was determined using Azomethine-H method suggested by Bingham (1982).

The weather data were obtained from Agro Meteorological Field Unit (Indian Meteorological Department), Agricultural Research Station, SKRAU, Bikaner. In all cases analyses were performed in triplicate and values averaged. A randomized complete block design was used in this experiment. Data obtained for two years were pooled as they showed similar trend during the studied period and were analyzed statistically using analysis of variance (ANOVA). Significance of difference among the treatments effects was tested through 'F' test and critical difference were calculated wherever the results were significant (Panse and Sukhatme 1989). Correlation between rising temperature, evaporation and incidence of stylar end browning were computed.

RESULTS AND DISCUSSION

The disorder affected fruits of Indian jujube cv. Chhuhara were similar in appearance and weight to normal fruits except to the presence of 'brown tip or stylar end' (Table 1, Fig 1). Both fruit skin and underlying tissues in pulp get affected due to this disorder. The appearance of a fresh fruits is considered to be the attribute most immediately obvious to the consumer, and strongly affects the decision to buy (Asioli *et al.* 2016); therefore, presence of brown fruit tip reduces acceptability of affected fruits. Furthermore, affected fruits had less TSS, reducing and total sugars; besides reduced ascorbic acid (Table 1).

The disorder 'stylar end browning' of Indian jujube cv. Chhuhara has been found to aggravate with the progress in the maturity. The size of affected area on stylar end increases with the maturity of fruits. Several disorders in fruit crops had been found to be associated with fruit maturity, e.g. disorders like soft scald, soggy breakdown, bitter pit, and senescent breakdown in apple has also been found to be more in late picked fruits of apple and generally increases with fruit maturity (Kumar and Kumar 2016).

Table 1 Physico-chemical characteristics of healthy and disorder-affected fruits of *ber* cv. Chhuhara

Characters	Healthy fruits	Disorder-affected fruits	CD (P=0.05)
Fruit length (mm)	35.2	35.4	NS
Fruit width (mm)	29.9	29.6	NS
Fruit weight (g)	22.2	21.8	NS
TSS (^{0} Brix)	15.7	14.3	0.71*
Reducing sugars (%)	8.9	7.6	0.69*
Total sugars (%)	14.6	12.7	1.36*
Acidity (%)	0.6	1.0	0.16*
Ascorbic acid (mg/100g)	89.1	44.8	13.6*

Fig 1 Stylar end browning in *ber* cv. Chhuhara (Left: healthy fruit; Right: affected fruits)

Browning in fruit crops are usually associated with the deficiency of nutrients like calcium (Ca) and boron (B) and in some cases with phosphorus (P) and potassium (K) (Franck *et al.* 2007, Buts *et al.* 2016, Kumar and Kumar 2016). Therefore, the contents of these nutrients were estimated in *ber* fruits. Upon nutrient analyses, it has been found that there were no significant differences in the contents of phosphorus, calcium and potassium in disorder affected and healthy fruits. On the other hand, the disordered fruits had higher accumulation of boron (Table 2).

Boron absorption by plant roots is affected by various environmental factors such as high temperature and evaporation, which are known to increase transpiration and in turn increase its uptake by roots (Camacho-Cristóbal *et al.* 2008); thereby, probably resulting in an increase in B uptake by *ber* cv. Chhuhara. Illumination has the same effect as increased temperature and as a result transpiration increases (Hu and Brown 1997) the B uptake. Under field conditions, other stresses such as low water availability and salinity may similarly intensify the toxicity symptoms of high B (Camacho-Cristóbal *et al.* 2008).

Though the harvesting of Indian jujube cv. Chhuhara lasts from the 2nd to 4th week of February, the brown tip disorder is more pronounced during late harvesting period

Table 2 Comparison of the contents of different nutrients in healthy and affected fruits of Indian jujube cv. Chhuhara

Nutrient	Nutrient contents in fruits		CD (P=0.05)
	Healthy fruits	Disorder-affected fruits	
Phosphorus (%)	0.200a	0.201a	NS
Potassium (ppm)	81.2a	79.2a	NS
Calcium (ppm)	178a	180.5a	NS
Boron (ppm)	18.7b	30.8a	4.13*

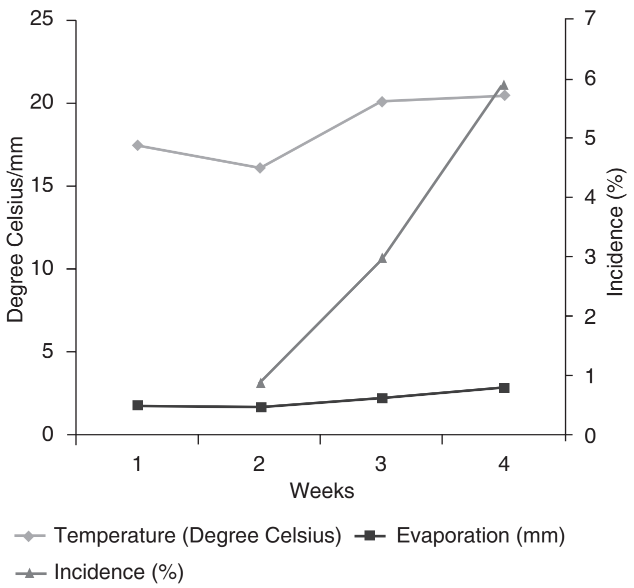

Fig 2 Weekly average temperature, evaporation and incidence of stylar end browning noted in Indian jujube cv. Chhuhara based on observation in February, 2014 and 2015

(end of 3rd week to 4th week of February) when average atmospheric temperature is higher, i.e. above 15°C under Bikaner conditions (Fig 2). However during the study period, the weekly average maximum temperature was in between 22-30°C (data not shown). In addition, evaporation also tends to increase with the passage of time during the aforementioned period. Both rising temperature ($r = 0.835$; $P<0.01$) and evaporation ($r = 0.966$; $P<0.01$) showed high positive correlation with incidence of stylar end browning in Chhuhara, which indicates that these climatic variable are important contributors to the development of physiological disorder in *ber* fruits. During this period, the daily solar radiation (from 3.60 in January to 4.14 kWh/m^2/d in February; Source: NASA Surface Meteorology and Solar Energy, https://eosweb.larc.nasa.gov/sse/) also increases as illumination period increases with the onset of spring. Therefore, it may be concluded that higher average temperature (above 15°C) and evaporation accompanied with high solar radiation had resulted in boron accumulation beyond its threshold level, which might have led to the development of browning of stylar end of fruits in *ber* cv. Chhuhara.

In some species, B is phloem-mobile and accumulates in developing sinks rather than at the end of the transpiration stream and thus the symptoms of toxicity are fruit disorders such as gummy nuts and internal necrosis (Hu and Brown 1996). In Chinese jujube (*Ziziphus jujube*) as well, phloem unloading has been noted to play a central role in photoassimilate transport and partitioning into sink organs (Nie *et al.* 2010). This assimilate transport system might also be taking place in Indian jujube as both Indian and Chinese jujube belong to the same genera. Therefore, accumulation of B, beyond a threshold level, in developing reproductive sink (fruits of Indian jujube) might have caused its toxicity, which manifested itself as stylar end browning. Besides, B accumulation follows a pattern from leaf base to tip in many plants and this leads to typical toxicity symptoms, which may appear as tip chlorosis or necrosis (Roessner *et al.* 2006). Presence of brown tip (stylar end) on fruits could be similar to the B accumulation pattern being followed in leaves.

The secondary metabolites like polyphenols and enzymes like PPO and POD, MDA, antioxidant activities, which are primarily associated with stress in plant system, have been found to be significantly higher in affected fruits (Table 3). Usually, browning in fruit results from the oxidation and polymerization of phenolic compounds and also involves the activities of PPO and POD. The MDA concentration was measured in *ber* fruits as an indicator of oxidative stress. FRAP and CUPRAC were used to provide information on total non-enzymatic antioxidant activity. Thus, browning of stylar end of *ber* fruits could be owing to accumulation of secondary metabolites and higher activity of enzymes, which are largely responsible for browning of plant tissues (Krishna *et al.* 2008). The increase in the activities of these compounds in affected *ber* fruits could be seen as a plant's defense mechanism to counter the B toxicity (Cervilla *et al.* 2012).

Browning of fruits has been associated with their poor quality and shelf life (Sun *et al.* 2009) as among the biochemical parameters which can affect the cultivar's suitability for storage and processing, the most significant are the activity of the key enzymes such as PPO and POD involved in the browning and senescence processes (Cefola *et al.* 2012). The disorder affected *ber* fruits had shorter economical shelf-life (4 days) as compared to normal fruits (6 days) under ambient conditions.

The reason behind report of stylar end browning only in Indian jujube cv. Chhuhara could be attributed to the fact that the varieties may differ in their genetic background to respond to B excess (Cervilla *et al.* 2012) and may differ in

Table 3 Contents of phenolic compounds, antioxidant and enzymatic activities in healthy and disorder-affected fruits of *ber* cv. Chhuhara

Characters	Healthy fruits	Disorder-affected fruits	CD (P=0.05)
Total phenolics (mg/100g FW)	101.7	286.3	24.75*
o-Dihydric phenol (mg/100g FW)	16.6	21.3	0.94*
Total flavonoids (mg/100g)	95.4	241.4	21.89*
Flavanol (mg/100g)	48.9	83.4	7.64*
Total antioxidant activity (CUPRAC, µmol TE/g)	4.61	7.34	0.47*
Total antioxidant activity (FRAP, µmol TE/g)	1.06	2.75	0.39*
DPPH inhibition (%)	75.9	96.6	8.36*
MDA value (nmol/g)	2.5	4.8	0.62*
PPO activity (unit/min./g)	57.8	453.4	36.08*
POD activity (nmol/min./g)	103.9	218.2	32.97*

their abilities to counter its ill effects. Though recent studies have improved our understanding of plant responses the mechanism by which plants respond to excess B (Martínez-Cuenca *et al.* 2015), it is still not sufficiently understood. Therefore, further investigation is needed to discern the mechanisms underlying response to B excess.

In conclusion, our study has shown that delay in harvest, especially after mid-February, may result in manifestation of boron-related disorder 'stylar end browning' in 'Chhuhara' *ber*. Therefore, this variety may be harvested at an optimum stage of fruit maturity when fruit are of sufficient size and appearance (typical of this variety) to meet market requirements with minimum risk of disorder incidence. Further, an understanding of pre-harvest factors such as characteristics of the fruiting site, nutrition of the developing fruit, temperature, water and gaseous relations, in the backdrop of climate change, will be necessary for the development of methods for predicting disorder risk.

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