

## Antioxidative responses of mandarin plants to water stress

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### Abstract

*Citrus reticulata* grows in warm climatic conditions. Stresses such as water logging, drought, soil acidity, unbalanced nutrition and pathogenic infestation lead to root injury resulting in citrus tree decline and therefore huge economic losses. In order to determine the effect of flooding and drought on mandarin plants, mandarin plants were subjected to water logging condition in the field and in pots for drought condition. Biochemical and morphological changes induced by water logging and drought conditions were determined. The plants showed slight wilting and leaf dropping by the third day of flooding whereas in drought the leaves curled up, became crisp and later dried out. Marked changes in antioxidative enzymes such as peroxidase, catalase and ascorbate peroxidase was observed during stress in comparison to control plants. Antioxidative activity was seen to be more in the leaves than in the roots. Among the antioxidants carotenoid content showed a significant decrease during the flood stress but increased in drought stress. An increase in ascorbate content was observed during stress in comparison to the control. From this study, we can conclude that water stress causes adjustment of antioxidant balance in mandarin plants.

**Keywords:** Mandarin, drought, flood

Water stress in citrus reduces stomatal conductance, transpiration rate and net assimilation of carbon dioxide (Arbona *et al.*, 2005; Garcia-Sanchez *et al.*, 2007). Waterlogging has a profound effect on root metabolism, mainly through the deficiency of oxygen (Drew 1997). In tropical and subtropical regions, severe crop losses are caused by prolonged seasonal rainfall. Previous work has shown that there is potential for water damage to citrus trees if roots are submerged in water for four days or more during frequent extended summer rains. During the cooler months of December through February, citrus trees can tolerate flooded conditions for longer periods than during the hot summer months. (Boman and Tucker, 2002). Sour odour in roots indicate an oxygen deficient environment. The presence of hydrogen sulfide (a rotten egg odour) is an indication that fresh feeder roots are dying. Anaerobic bacteria (which grow only in the absence of oxygen) develop rapidly in flooded soils and contribute to the destruction of citrus roots. Root damage symptoms include leaf yellowing, chlorosis, wilting, fruit drop, leaf drop and dieback. Excess water produces anoxic soil conditions within a few hours (Gambrell

and Patrick, 1978). Flooding stress is usually less when water is moving than when water is stagnant for anaerobics cannot multiply if oxygen is present.

Flooding will also affect many other biochemical and physical processes taking place in the rhizosphere (He *et al.*, 1996). Flooding of the root system arrests growth and diminishes the productivity in the majority of terrestrial plants (Limkemer *et al.*, 1998).

Drought stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Desiccation is much more extensive loss of water, which can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme catalyzed reactions (Smirnoff 1993; Jaleel *et al.*, 2007).

Drought limits canopy development and inhibits vegetative and fruit growth. In many plant species, root hydraulic conductance decreases significantly under water deficit situations (Sumner & Boswell, 1981; Cruz *et al.*, 1992; North and Nobel, 1996; North *et al.*, 2004). Plants have also developed various mechanisms such as higher root-shoot ratios, fewer and smaller leaves, concentrated solutes or increased activity of oxidative stress

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enzymes in leaf cells to withstand water stress (Lei *et al.*, 2006).

Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al.*, 2008)

Drought is one of the most common environmental limitations affecting growth and productivity of plants, and causes many metabolic and oxidative changes in plants (Reddy *et al.*, 2004) and recent global climate change has made this situation more serious (Pan *et al.*, 2002)

Considering the importance of water stress in mandarin the present work was undertaken to determine the effects of flooding and drought stress in mandarin plants.

## Materials and Method

### Plant material

Four year old mandarin (*Citrus reticulata*) plants were obtained from Kalimpong, Mirik, Bijanbari and Teesta Valley orchards. These were then maintained in the experimental plot of the Department. One set of plants were maintained in the field whereas another set was planted in pots.

### Water stress treatment

For flooding, the plants in the field were flooded with water and flooding condition was maintained for desired period. In case of drought, potted plants were left without water for the desired period.

### Extraction and assay of enzyme activities

#### Preparation of enzyme extract

The leaves and roots collected from treated and control plants were ground to fine powder with a mortar and pestle under liquid nitrogen in cold using respective buffers containing 1% (w/v) polyvinylpyrrolidone. The homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was directly used as crude extract for enzyme assays.

### Assay of activities

#### Peroxidase (POX: EC. 1.11.17)

Peroxidase activity was assayed following the method described by Chakraborty *et al.* (1993) spectrophotometrically in UV VIS spectrophotometer (Model 118 SYSTRONICS) at 460 nm by monitoring the oxidation of O-dianisidine in presence of H<sub>2</sub>O<sub>2</sub>. Specific activity was expressed as "A<sub>460</sub> mg protein<sup>-1</sup> min<sup>-1</sup>."

#### Ascorbate peroxidase (APOX : EC.1.11.1.11)

Activity was assayed as decrease in absorbance by monitoring the oxidation of ascorbate at 290 nm according to the method of Asada and Takahashi (1987) with some modification. Enzyme activity was expressed as  $\Delta A_{290}$  mg protein<sup>-1</sup> min<sup>-1</sup>.

#### Catalase (CAT: EC.1.11.1.6)

Catalase activity was assayed as described by Beers and Sizer (1952) by estimating the breakdown of H<sub>2</sub>O<sub>2</sub> which was measured at 240 nm in a spectrophotometer. The enzyme activity was expressed as  $\Delta A_{245}$  mg protein<sup>-1</sup> min<sup>-1</sup>.

#### Phenylalanine Ammonia Lyase (PAL: EC.4.3.1.5)

Phenylalanine Ammonia Lyase was extracted and estimated following the method described by Chakraborty *et al.* (1993).

#### Proline

Free proline was extracted and estimated following the method of Bates *et al.* (1973). Fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 2 filter paper. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and absorbance was measured at 520 nm.

#### Protein

Protein contents in each case were extracted from leaves and roots of maize by following the method of Chakraborty *et al.* (1995) and estimated by following the method of Lowry *et al.* (1951).

#### Ascorbate

Ascorbate was extracted and estimated by

following the method of Mukherjee and Choudhuri (1983). The concentration of ascorbate was calculated from a standard curve plotted with known concentration of ascorbic acid.

#### Carotenoids

Carotenoids were extracted and estimated following the method described by Lichtenthaler (1987). Extraction was done in methanol and the extract was filtered. Absorbance of the filtrate was noted at 480nm, 663nm and 645nm in a VIS spectrophotometer and the carotenoid content was calculated using the standard formula.

### Results and Discussion

In the present study, citrus seedlings obtained from the four locations- Kalimpong (KL), Bijanbari (BJ), Mirik (MR) and Teesta Valley (TV) showed varying responses to flooding and drought (Fig. 1A & Fig. 1B). Among the four, the plants obtained from Bijanbari seemed to be more susceptible. Like other crops, citrus trees respond to flooding by reducing leaf water potential, stomatal conductance, gas exchange and plant growth (Vu and Yelenosky,

1991). During prolonged periods of soil flooding, a decrease in root hydraulic conductance (Ruizsanchez *et al.*, 1996; Syvertsen *et al.*, 1983) causes impairment of water uptake, which eventually leads to leaf wilting and chlorosis (Arbona *et al.*, 2008).

The antioxidant defense machinery protects plants against oxidative stress damages. Plants possess very efficient enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, guaiacol peroxidase, and glutathione-S-transferase) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (Gill and Tuteja, 2010).

Catalase (Fig.2A and B) and ascorbate peroxidase (Fig. 2C and D) activity decreased in almost all the samples during both types of stresses but peroxidase (Fig. 3A and B) activity mostly increased in leaf specially during both drought and

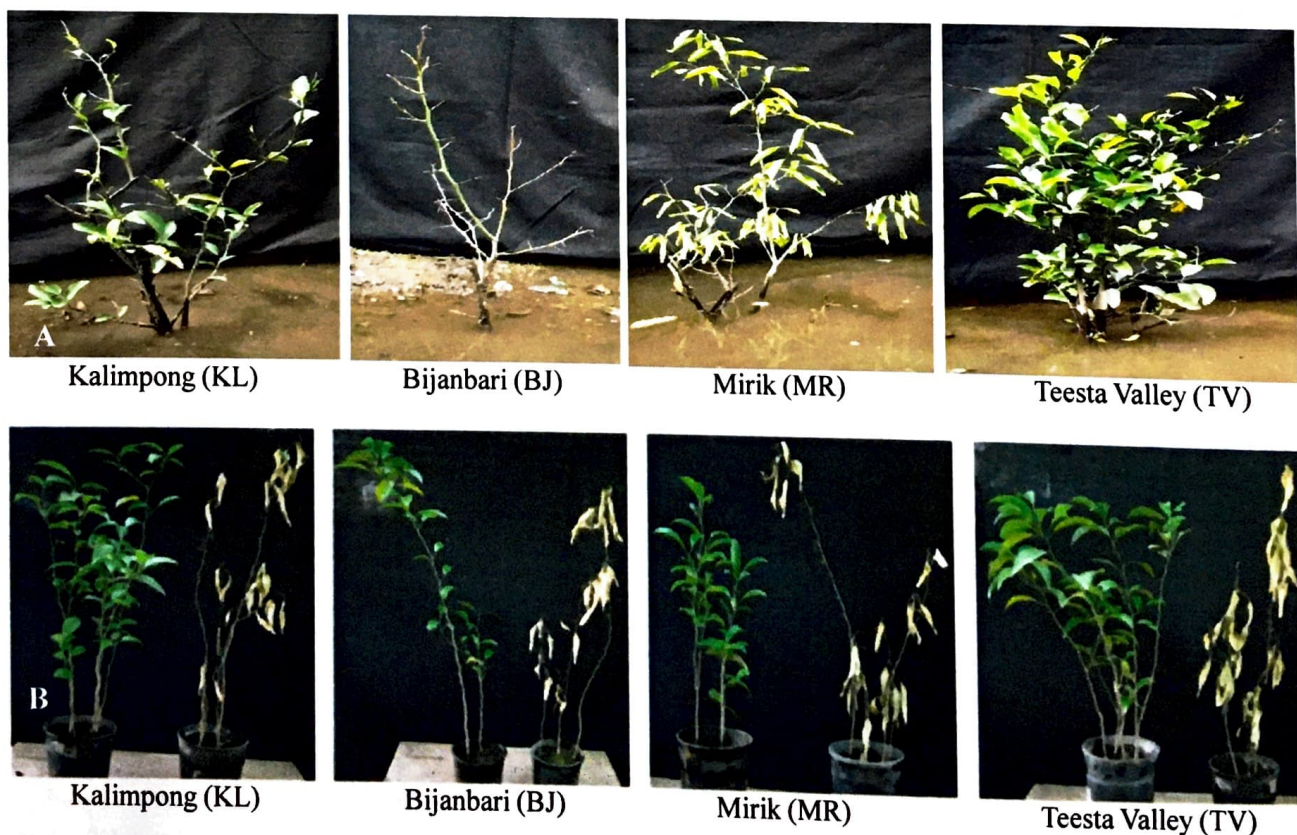


Fig. 1: Responses of mandarin plants from different locations to flood (A) and drought (B)

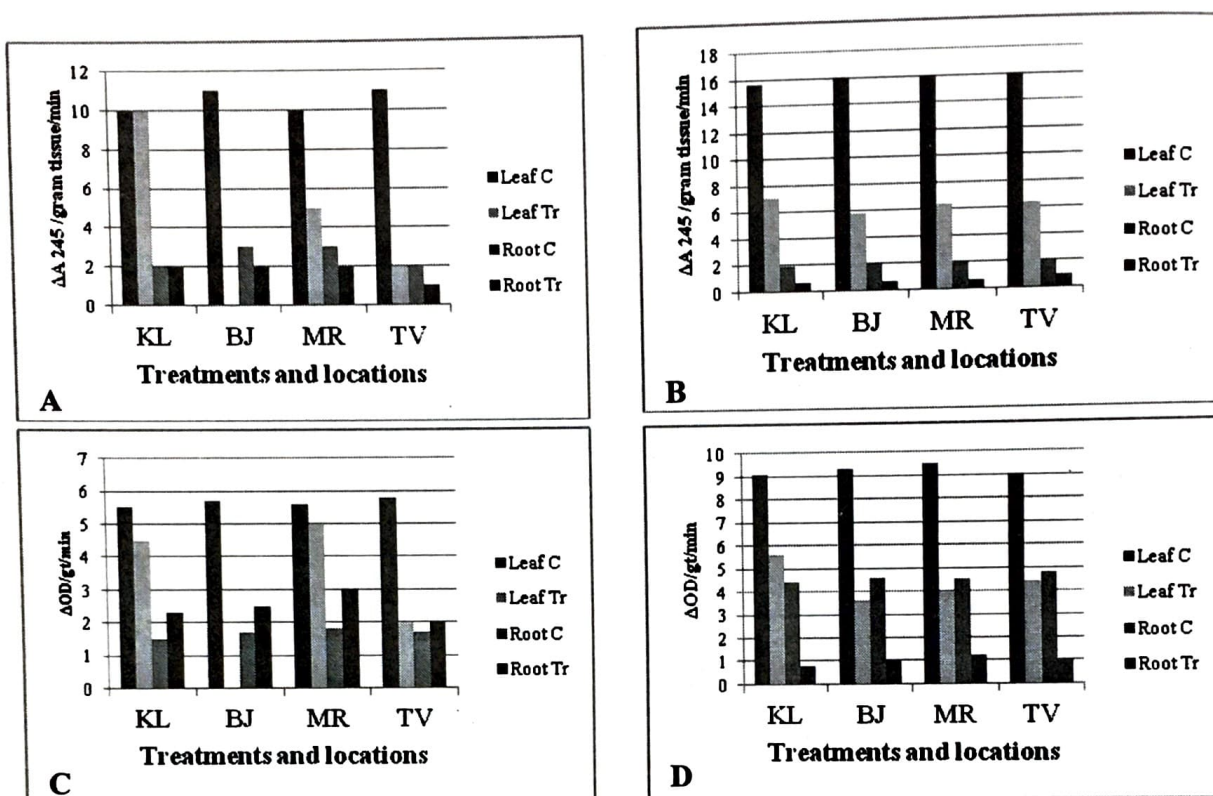


Fig. 2: Catalase (A&B) & Ascorbate peroxidase (C&D) activities during flooding (A&C) and drought (B&D)

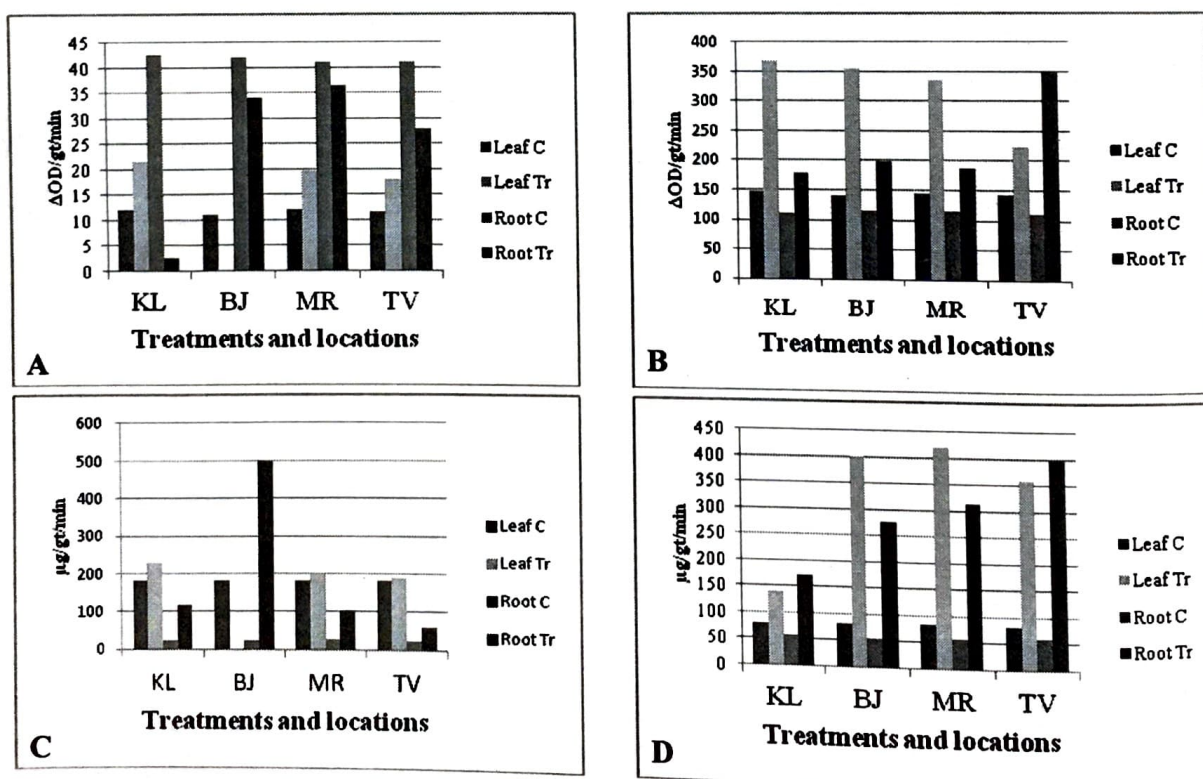


Fig. 3: Peroxidase (A&B) & Phenylalanine Ammonia Lyase (C&D) activities during flooding (A&C) and drought (B&D)

flooding stress indicating that the peroxidase activity plays a vital role in scavenging of free radicals during water stress in mandarin plant. It has been reported that catalase activity declined during stress in Dhanya and Swarna varieties of maize while the other two varieties showed an increase during drought stress (Lama and Chakraborty, 2012). Maintaining a relative higher antioxidants activity may lead to drought tolerance by improving the capacity to cope with ROS (Sharma and Dubey, 2005).

PAL activity showed varied trends in samples subjected to flooding stress, the activity increased in some samples during stress in both leaf and root

whereas in some samples the activity decreased with flooding stress (Fig. 3C). But in case of drought (Fig. 3D) the activity increased in both the leaf and the root of the samples in comparison to the control plants. Maximum activity was observed in BJ, MR and TV in both the leaf and roots of these samples.

Ascorbate content showed an increase in both flooding and drought stress in all the samples of mandarin (Table 1) but carotenoid content showed a different trend in two type of water stress where a decrease in the carotenoid content was observed in flooding (Table 1) whereas there was an increase in carotenoid content during drought. Accumulation of ascorbate and carotenoids was enhanced

Table 1: Ascorbate and carotenoid content during flooding and drought

Plant	Ascorbate ( $\mu\text{g/g}$ tissue)				Carotenoid ( $\text{mg/g}$ tissue)			
	Drought		Flood		Drought		Flood	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
KL	4	6.50	9.26	10.78	0.006	0.025	0.027	0.026
BJ	4.11	7.75	9.23	0.000	0.005	0.010	0.027	0.000
MR	4.15	7.64	9.11	18.70	0.002	0.017	0.027	0.016
TV	4.19	7.30	9.12	14.32	0.004	0.018	0.027	0.020

significantly following water stress in both the varieties, but it was greater in variety 30P30 (Chakraborty and Lama, 2010).

Protein content in all the samples was more in the leaves than in the root during both drought and flooding stress (Table 2). Under anoxia conditions, the pattern of protein synthesis is altered in plants. The proteins which are synthesized as a specific response to anaerobes are called anaerobic polypeptides (ANPs) (Sachs *et al.*, 1980).

During drought stress proline (Table 3) content

increased in all the four samples of mandarin plant. Both leaves and root showed an increase during drought stress but there was more increase in the roots than in the shoots. Proline which is known to occur widely in higher plants, normally accumulated in large quantities in response to environmental stresses (Kavikishore *et al.*, 2005; Ashraf and Foolad, 2007). Accumulation of osmolytes such as proline, helps in maintaining cell water status, sub-cellular structures and protecting membranes and proteins from the denaturing effects of the osmotic stress (Ashraf and Foolad,

Table 2: Protein content of leaf and root during flooding and drought

Plant	Protein (Leaf) ( $\text{mg/g}$ tissue)				Protein (Root) ( $\text{mg/g}$ tissue)			
	Drought		Flood		Drought		Flood	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
KL	150.5	192.0	235	182.5	15.0	26.0	55.0	70.0
BJ	150.5	200.0	235	0.000	15.0	24.0	55.0	75.0
MR	150.5	196.0	235	225.6	15.0	24.0	55.0	62.5
TV	150.5	196.0	235	200.0	15.0	26.0	55.0	50.0

Table 3: Proline content of leaf and root during drought

Plant	Proline (Leaf) (mg/g tissue)		Proline (Root) (mg/g tissue)	
	Control	Treated	Control	Treated
KL	0.56	0.59	0.9	1.34
BJ	0.5	1.23	0.96	1.76
MR	0.52	1.94	0.96	2.12
TV	0.51	1.52	0.94	3.36

2007). With the increase in intensity of drought there was an increase in both proline and ascorbate content in all varieties of maize (Lama and Chakraborty, 2012).

PAGE analyses was performed in plant sample subjected to drought stress and new bands of isoform was observed in leaf and root of plant sample subjected to drought stress (Fig. 4). Being a self-regulating system, the plant is capable of developing a survival strategy under stressful conditions. It should be borne in mind that the action of stress factor on the underground plant part causes a series of physiological changes in the aboveground organs; these changes reflect either plant adaptation or damage (Krishtetter *et al.*, 1998). As a result of the above study we can conclude that mandarin plants do have the ability to cope with both kinds of stress to some extent.

It is known that plant adaptation to stress conditions requires additional material and energy resources,

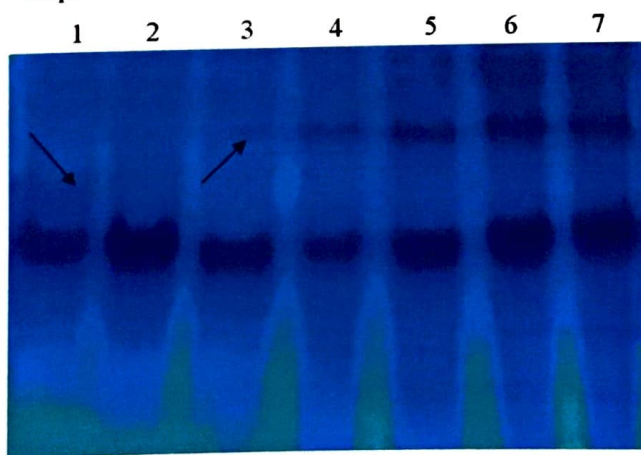


Fig 4: Isozyme analysis of peroxidase from leaves and roots of mandarin. 1- Control leaf; 2 & 3- Drought treated leaves - KL & BJ, respectively; 4 Control root; 5-7 Drought treated roots - KL, BJ & MR respectively.

making processes such as photosynthesis and respiration more significant (Semikhatova 2000). Soil water logging and submergence (collectively termed flooding) and drought are abiotic stresses that influence species composition and productivity in numerous plant communities, world-wide. Hydrological patterns can determine the vegetation in natural and man-made wetlands, since this is dependent on ecophysiological responses of species to flooding (e.g. Voesenek *et al.*, 2004). For most other crops, excess water is a major constraint which adversely affects grain yields (Setter and Waters, 2003) and growth of pasture species (Gibberd and Cocks, 1997; Gibberd *et al.*, 2001). Drought triggers a wide variety of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions (Anjum *et al.*, 2011).

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