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Flood induced adventitious root formation and ethylene accumulation in *Solanum* species

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Abstract
The frequency of extreme events such as droughts and floods has increased as a consequence of climate change. Many crops have not been improved to tolerate soil anoxia and, therefore, floods cause important economic losses. During waterlogging, *Solanum* species exhibits three distinct responses which are adventitious root production, ethylene production and aerenchyma formation. The development of a new adventitious root system is crucial as it can replace the original roots that succumb to the hypoxic environment. *Solanum* torvum is a wild *Solanum* species characterized by the presence of numerous adventitious root along with aerenchyma. In this work, we have analyzed the *Solanum* species to flooding to determine flood tolerance and to use them as a rootstock for future breeding programme. We have also examined them for other character viz., aerenchyma formation, ethylene, and adventitious roots plants to detect differential adaptations under flooding. *Solanum* torvum form an abundant adventitious root system faster than other *Solanum* species, which results in tolerant species accumulating as much biomass as susceptible plants. In addition, several ethylene-induced responses such as epinasty, aerenchyma production, confirms the presence of ethylene during the flooding. Therefore, our results indicate that the rapid formation of a new root system together with ethylene is responsible for faster adaptation to flooding stress in *Solanum torvum*.

Keywords: Ethylene, arenchyma, *Solanum*, adventitious root

Introduction

Solanaceous vegetables are nutritionally rich and high valued crops. These vegetables are highly sensitive to climate change and any sudden variation in the weather condition at any growth stage will affect the normal vegetative flowering and reproductive stages and hence the yield of the crop. Changes in climatic conditions possess increase in the occurrence of heavy rains in tropical and subtropical regions leading to flooding of agricultural lands. Currently flooding has become an important global crop production constraint causing significant yield reduction in several crops. Flooding is one among the abiotic stresses causing crop loss due to excessive or uncertainty in rainfall (Oh *et al.*, 2014) [1]. The change in endogenous production of ethylene and nitric oxide are some of the indicators which express the efficiency of water logging in terms of production and formation of adventitious roots and aerenchyma. Flooding is one of the critical abiotic stress factors which has a considerable impact on crop growth, eventually leads to decline in the yield and production of various crop (Normile 2008) [2]. Due to flooding plants does not receive little (hypoxia) and no oxygen (anoxia) leading to poor oxygen level that will affect the respiration and prevent the root growth that in turn reduce the shoot growth and plant productivity of vegetable crops particularly tomato (*Solanum* species). In tomato ethylene was accumulated during flooding condition causing severe damage to the crop (Drew *et al.*, 1979) [3]. It was indicated that the decrease in yield was attributed to decrease in nitrogen uptake and reduced photosynthesis under waterlogging. The most common morphological response of plants during flowering is the development of adventitious roots which may appear in the hypocotyl region of the plant. Besides the development of adventitious roots, the root aeration was channelized through the formation of aerenchyma for the sustenance of the plant (Zhang *et al.*, 2017) [4]. Tomato is considered to be one of the most sensitive vegetable species to excessive soil moisture. Speculating the problem of flooding condition in future, alternate tool to mitigate stress can achieve through grafting.

Grafting has evolved as an promising technique to withstand the global climate change generated by biotic and abiotic stresses. It offers an opportunity to use rootstocks of the specific traits to have its influence over the scions phenotype. Selection of tolerant plants under waterlogging condition is the indicator that can be used as the rootstock for the production of water logging resistant plants. Hence the present study undertaken to screen *Solanum* species for flooding tolerance through morphological characterization.

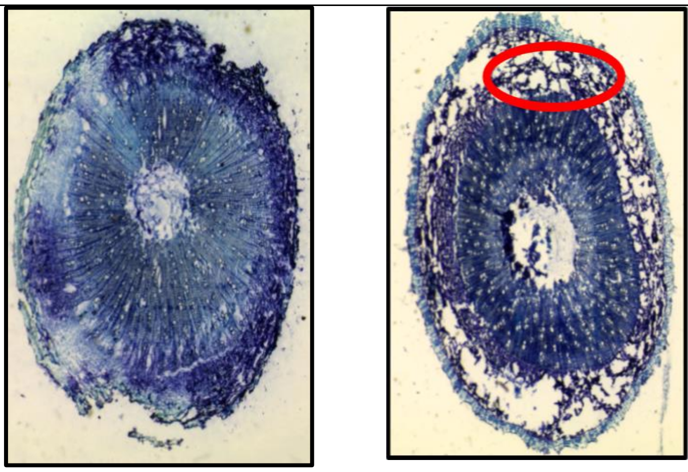
Materials and Methods

Nine *Solanum* species used for this study were the recommended dose of fertilizer and common package of practice were followed in timely fashion. Seeds of nine *Solanum* species were sown in portrays and transferred to submergence tank containing red soil. Environmental conditions, watering regime, and imposition of flood treatment were as described by McNamara and Mitchell (1989) [5]. Flood treatment was imposed by placing seedling into submergence tank. Plants were immersed to 3 cm above the soil surface. Control plants were placed within similar containers containing bottom-drainage holes, and were watered to field capacity once daily.

Ethylene were estimated for all the species by using gas chromatography and the instrument specialized with flame ionization detector and a 2-mm-diameter (I.d.)×182-cm-long column packed with alumina. chromatography oven temperature were 80 °C for column 120 °C for injector and 150 °C for detector and flow rate of nitrogen carrier gas was 40 ml min^{-1} By the way samples of 2.5 cm long hypocotyls excised after 48 h of treatment weighed and enclosed in 5 ml vials later the 1 ml gas samples was withdrawn and injected into gas chromatography as described by Mcnamara and Mitchell (1990) [6]. Adventitious root formation was counted on the basis of number of roots produced above soil surface after imposing flooding for 7 days. The no of adventitious roots were recorded at the 7th day of flooding. For histological studies, hypocotyl was sectioned after 7 days of flooding and was fixed using a solution containing formalin, glacial acetic acid and 95% ethanol in the ratio 5:5:90 then it was passed through an ethanol-tertiary butanol dehydration series, and embedded in Paraplast (Sass, 1958) [7]. Sections taken were 10 µm thick and were stained with safranin/ aniline blue.

Results and Discussion

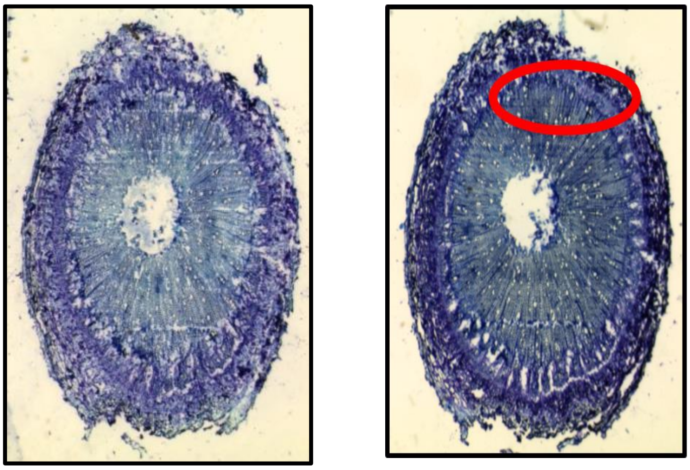
Gas diffusion into the plant is impeded, leading to low oxygen partial pressure that stimulates biosynthesis of ethylene by increasing ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity and further enriching the stem with ethylene (Sauter, 2013) [8] and also inducing adventitious root and aerenchyma tissue formation. Hormonal balance is an adaptive response to flooding condition by the regulation of increase in ethylene concentration, which interacts with gibberellins (Musgrave *et al.*, 1972) [9] and auxins. In this study based on their performance in flooding and with their morpho physiological, biochemical parameters the most susceptible and the tolerant species were identified and ethylene was determined in those species. Flooding leads to the increase in the concentration of ethylene in the hypocotyl region. Ethylene estimation was done for the selected tolerant and the susceptible species and the amount of ethylene content varied between these species. The *Solanum* species recorded higher concentration of ethylene *S. aculeatissimum* (1.65 nl/ g of fresh weight/ hr) followed by *S. torvum* (1.012 nl/ g of fresh weight/ hr) and *S. sisymbriifolium* (0.986 nl/ g of fresh weight/ hr) under the flooding condition and the lowest ethylene content was recorded in *S. viarum* of (0.02 nl/ g of fresh weight/ hr) (Plate 2). The ethylene production in the shoot tissue will be stimulated because of the anoxia condition (Steffens *et al*., 2011) [12] that will encourage cell separation and development of air spaces in their stem (Khah, *et al*., 2012) [10]. The acceleration of air spaces formation in the newly produced nodal roots of zea maize has some feature in common with swelling of preformed stems in sunflower induced by ethyphon or flooding. The present study revealed that flooding affected the rate of ethylene evolution from hypocotyls of the different species. The hypocotyls of the flooded *Solanum* species *Solanum aculeatissimum* (1.652 nl/ g of fresh weight/ hr) (Fig 3) produced significantly higher ethylene when compared to control. Studies by Bellini *et al*., 2014 [11], indicated that the restricted capacity of susceptible species for Arenchyma and adventitious roots development limited by its ability to synthesize ACC or C_2H_4 during inundation. These studies support for the present findings where significant increase in the number of adventitious roots. In *S. torvum* than in other *Solanum* species were the ethylene correlate with adventitious root numbers (Plate.1) (Fig 1). As from the previous study adventitious roots development was not found in control condition and it was significantly higher under flood imposed treatment. The number of adventitious ranged from 17-52 per plant (fig 3). *S. torvum* recorded significantly higher number of adventitious roots 52 followed by *S. aculeatissimum* 36. In some of species like *S. nigrum, S. macrocarpon,* there was no development of adventitious root. The microscopic thin section of the hypocotyl tissue of different species revealed that hypocotyl porosity was increased under flooding condition. The aerenchyma development in terms of porosity was more in *Solanum torvum* 1(a) 2(a), *Solanum macrocarpon, Solanum aculeatissimum* and *Solanum sisymbriifolium* and there was no development of aerenchyma in *Solanum nigrum* 1(b), 1(b).



1(a) Hypocotyl cross-sections of *Solanum torvum* seedlings control 1

1(b) 7days after flooding 1

Fig 1(a-b): Red circle showing absence of adventitious or. Magnification.



2(a) Hypocotyl cross-sections of *Solanum nigrum* seedlings control

2(b) 7days after flooding

Fig 2(a-b): Showing absence of adventitious root primordia. Magnification.

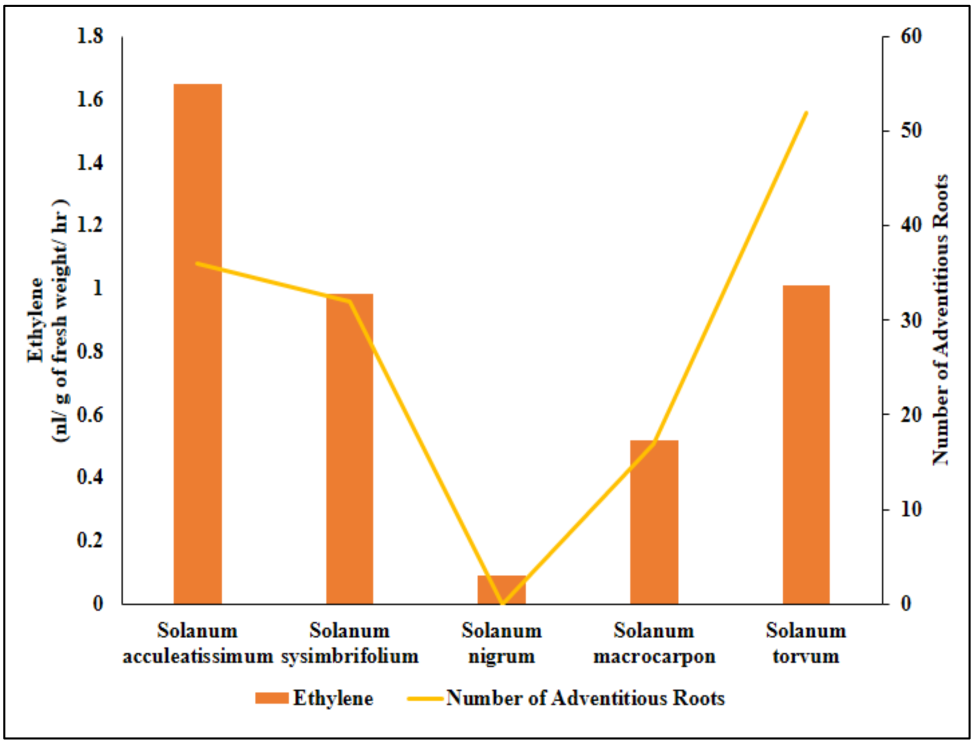


Fig 3: Effect of flooding on ethylene and adventitious root formation

Plate 1: Adventitious root formation in tolerant species.

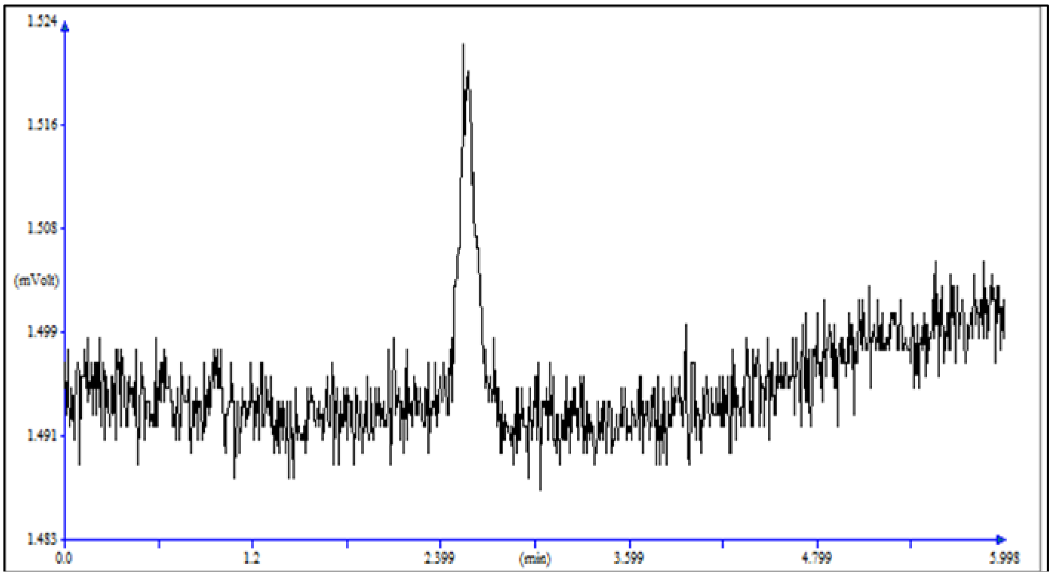


Plate 2: Ethylene peak observed in gas chromatography of tolerant species.

Conclusion

Clear evidence is emerging demonstrating that the adventitious root and arenchyma formation is regulated and responds to environmental cues. Because adventitious roots are important for tolerance to flooding. In this study the hormonal interaction and formation of adventitious roots and aerenchyma formation is more evident. The formation of aerenchyma in adventitious roots and in the hypocotyl, region is common in flood tolerant species. It is concluded S. torvum is suitable for mother stock flood susceptible solanaceous vegetables. In further Physiological and molecular analysis in combination with genetic modification are planned to shed further light on the roles of the various signalling pathways.

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