RESEARCH PAPER | Agriculture | Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

# Physio-Chemical and Physiological Reactions of Waterlogging Stress on *Aloe Vera L.* Genotypes

KEYWORDS: Oxygen transport, ATP, Chlorophyll fluorescence, Photo system II, and Reactive Oxygen Species.

**Dr. G. Alagukannan**
Faculty of Agriculture, Gandhigram Rural University, Dindigul, Tamilnadu, Pin- 624 302

**Dr. S. Ganesh**
Professor of Horticulture, Faculty of Agriculture, Gandhigram Rural University, Dindigul, Tamilnadu, Pin- 624 302

**ABSTRACT** *Aloe vera is extensively used in preparation of medicines, cosmetics and food supplements. In order to identify the Aloe vera genotype suitable for commercial cultivation in TamilNadu, a study was conducted at Gandhigram Rural University, Tamilnadu with 21 genotypes. Due to the heavy downpour of rainfall continuously for 96 hours and thereby waterlogging the lower two-three leaves of genotype IIHR $AV_{15}$ started to wither from its tip to downward. The root system of the affected plants was blackened and bark peeled off with the formation of adventitious roots. The reasons viz., hindrance in gaseous phase of soil and oxygen transport, stomatal disfunctioning, perturbation in chlorophyll fluorescence, oxidative damage by reactive oxygen species, changes in soil $p^H$ and evolution of toxins were discussed. Among the genotypes studied, except IIHR $AV_{15}$ all other genotypes can be potentiality utilized for future breeding programmes aiming at waterlogging stress.*

**Introduction**

A conservative estimate puts the monetary value of current global trade in medicinal plants at over US $ 60 billion. With the increasing interest in natural products across the world the demand for medicinal plants, this trade is expected to grow upto US $ 5 trillion by 2050. *Aloe vera* is among the few medicinal plants by virtue of its extensive medicinal, nutraceutical, cosmetic and other uses enjoy a major chunk of the market across the world (Yoheeswaran et al., 2005). *Aloe vera* plants yields two distinct commercially important products viz., Aloe juice/latex and gel. The main constituents of latex are anthraquinones including hydraxyanthracene derivatives, Aloin A and B, Isobarbaloin and Emodin. Aloe gel is the colourless mucilaginous substance obtained from the paranchymatous cells of fresh leaves of Aloe contains mono and polysaccharides and enzymes responsible for the curing effect of different diseases of human being and also to boost the immune system.

India is gifted with the unique geographical features essential for the cultivation of Aloe vera. But so far its commercial cultivation is not popular among the farming community and it is widely collected from the wild source. The reasons are lack of technical know-how on correct type of Aloe vera and its cultural hints. Though there is much diversity in Aloe vera, the cultivators unable to choose the best genotype for commercial cultivation. Drought and waterlogging are the two major obstacles for crop production in tropical and subtropical regions. Plants subjected to waterlogging suffer from substantial yield loss. Waterlogging includes a number of alterations in important soil physio-chemical properties like $p^H$, Redox potential and oxygen level.

Hence, a study was initiated during Sep, 2005 and completed during January, 2011 at Faculty of Agriculture, Gandhigram Rural University, Gandhigram, TamilNadu on various aspects of Aloe vera. The first phase of study was conducted to evaluate the different genotypes of Aloe vera available in the southern parts of India for its withstanding ability of waterlogging, drought resistance and genetic variability and to identify the promising types. In this paper an attempt is made to bring out the deleterious effects of waterlogging in Aloe vera and to suggest the suitable strains for further breeding programmes.

**Materials and Methods**

Fifty two genotypes of Aloe vera were collected from different parts of South India from the wild state and also from research institutions such as Indian Institute of Horticultural Research, Bangalore, TamilNadu Agricultural University, Coimbatore, Pandit Jawaharlal Nehru College of Agriculture, Karaikal and Jawaharlal Nehru Institute of Post Graduate Medical Education and Research, Pondicherry. Wild types were also collected from the adjacent states such as Kerala and Andhrapradesh. After purging the identical ones, twenty one genotypes were considered for further study. They were named shortly after the place of collection. To evaluate these twenty one types they were planted in Randomized Block Design (RBD) replicated twice. The soil type of the experimental plot was red loamy with the $p^H$ of 7.5 and electrical conductivity of 0.14 $dsm^{-1}$. The suckers of about 15-18cm height were planted in beds and channels at a spacing of 60 X 45 cm. The intercultural operations like irrigation, weeding were done periodically.

**Results**

At third month after planting, an event of heavy downpour of rainfall has occurred during North-East monsoon and the total quantum of rainfall received was 232.4 mm over a period of 96 hours (Table.1). The Tensiometers placed at 15 and 30cm soil depth showed the water tension of zero and one centibar (cb) respectively for the period of day first of commencement of rainfall and continued upto 7 days.

**Table. 1. The quantum of rainfall received during the reporting period**

| Day | Date | Quantum of rainfall received (mm) |
|---|---|---|
| 1 | 22nd Nov,2005 | 20.0 |
| 2 | 23rd Nov, 2005 | 115.0 |
| 3 | 24th Nov, 2005 | 79.8 |
| 4 | 25th Nov, 2005 | 17.6 |
| | Total | 232.4 |

The morphological, anatomical features and relative performance of different genotypes studied are depicted in Table.2. There was a considerable variation in leaf colour of Aloe vera genotypes ranging from pale, light green, green and dark green. In all the genotypes tested it was observed that the stomata was closed during day and opened during night. But the stomata of genotype collected from Indian Institute of Horticultural Research, Bangalore named as IIHR AV15 remain closed during day and night and started showing the symptoms of withering.

Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

Further, the lower two - three leaves of IIHR $AV_{15}$ plants started to decay from its tip to downward and no disease causing pathogen was observed. This sudden response of IIHR $AV_{15}$ to waterlogging shows its susceptibility to the waterlogged condition and thereby suffocation even it is grown under sandy loam soil. The plants of other genotypes standing on the adjacent plots devoid of such decaying symptom and they were normal.

The appearance of symptoms was first noticed in the foliage. This includes yellowing and formation of soft tissues starting from leaf tip and slowly spreading towards the base. Eventually the whole leaf was blackened from tip to downward with high water content ie., water soaked leaves (Fig.1). The root system of the damaged plants was also examined and most of the roots were blackened and the bark peeled off. The minute adventitious root formation was observed in the plants of IIHR $AV_{15}$.


**Fig. 1. Plants of Aloe vera genotype (IIHR $AV_{15}$) affected by waterlogging replanted for rescue**

**Table. 2. Morphological and anatomical features of different Aloe vera genotypes under waterlogged condition**

| S. No. | Code name of the genotype | characters | | | | | | Susceptibility or tolerance to waterlogging |
|---|---|---|---|---|---|---|---|---|
| | | Leaf colour | Stomatal opening | | Leaf Moisture level | Symptoms expressed by waterlogged condition | | |
| | | | Day | Night | | On leaves | On roots | |
| 1 | SLM | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 2 | GGM | Green | Close | Open | Normal | Brown sunken spots | No Symptom | Susceptible to leaf spot disease |
| 3 | IIHR $AV_1$ | Light green | Close | Open | Normal | No symptom | No Symptom | Tolerant |
| 4 | IIHR $AV_2$ | Light green | Close | Open | Normal | No symptom | No Symptom | Tolerant |
| 5 | IIHR $AV_3$ | Light green | Close | Open | Normal | No symptom | No Symptom | Tolerant |
| 6 | IIHR $AV_4$ | Pale | Close | Open | Normal | No symptom | No Symptom | Tolerant |
| 7 | IIHR $AV_{13}$ | Green | Close | Open | Normal | No symptom | No Symptom | Tolerant |
| 8 | IIHR $AV_{15}$ | Dark green with red spines | Close | Remain closed | Very high | Blackening and drying of leaves from tip to base | Adventitious root formation above soil level. Drying and easy peel off of bark from feeder roots. | Highly susceptible |
| 9 | VNR | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 10 | KH | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 11 | KRR | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 12 | ODC | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 13 | KKL | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 14 | PY | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 15 | TCY | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 16 | HSR | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 17 | EDE | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 18 | CBE | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 19 | KK-AP | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 20 | TVM | Green | Close | Open | Normal | No symptom | No symptom | Tolerant |
| 21 | KL | Light green | Close | Open | Normal | No symptom | No symptom | Tolerant |

**Discussion**

Everyone presumes that Aloe vera is a hardy plant which can tolerate drought and perhaps it is also right as per waterlogging is concerned. Even then there is a degree of variation in withstanding ability of Aloe vera strains against waterlogged condition. Waterlogging stress is the major constraint in sustainable agriculture and a marked variation in genetic resources of potential crops like Wheat (Gardner and Flood, 1993) and Maize (Anjus e Silva et al., 2005).

The probable causes for the susceptibility and expression of varying symptoms of damage of Aloe vera genotype IIHR $AV_{15}$ for waterlogging is discussed below.

**Hindrance in gaseous phase of soil and oxygen transport**

The air filled pores constitute the gaseous phase of the soil system. The volume of gaseous phase is thus dependent on that the liquid phase. Water absorption by root cells is not a mere physical process involving only osmotic and imbibition phenomena but is a vital process which involves the expenditure of energy released in the process of respiration. Excessive soil moisture due to flooding could resulted in root tip death due to oxygen depletion in the root zone that situation is known as suffocation.

Root growth and maintenance of root tissue mainly rely on respiration (oxidative phosphorylation) as the most efficient sources of energy. Respiration however depends on the supply of oxygen. Oxygen that is consumed in plant tissues by respiration is eventually replenished by the oxygen reservoir provided by the atmosphere and by air-filled cavities in the soil. A phase transition of $O_2$ from the gas phase to the fluid phase (or vice versa), usually occurring in soil or at sites within plant organs (sub-stomatal cavities, gas filled intercellular spaces, lacunae, aerenchyma) is governed by Henry's law, provided that the two phases are approximately at equilibrium:

$$P(O_2) = k_H c_{O2} \qquad \text{(for T = const)}$$

stating that the oxygen concentration, $c_{O2}$, in solution is proportional to the partial pressure in the adjacent gas phase, $P(O_2)$; $k_H$ is Henry's gas constant for $O_2$ in water. The solubility of $O_2$ in water is relatively high; at the usual partial pressure of $O_2$ in air (0.2 bar), a concentration of 8 mg $O_2$ $l^{-1}$, corresponding to 0.25 mM, is calculated at T = 298 K. This means

Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

that does not predict any shortage of Oxygen in plants. Any imbalance in the equilibrium due to elevated levels of water on soil creates hypoxia or anoxia condition (Gambrell and Patrick, 1978). Oxygen deficiency inhibits the root respiration of plants which results in substantial reduction on energy status of root cells. Since oxygen is a terminal electron acceptor in aerobic respiration, in its absence Kreb's cycle and electron transport systems are blocked. Hence the plants are forced to use the alternate pathway of fermentative metabolism to produce ATP whereby plants could produce only two ATP molecules per glucose molecule whereas 36 ATP molecules are produced per glucose molecule in aerobic respiration. Further it is also resulting in enhanced accumulation of ethanol and increased activity of alcohol dehydrogenase (Vartapetian, 1991).

**Stomatal disfunctioning**

One of the first plant responses to waterlogging is the reduction in stomatal conductance (Folzer et al., 2006). Aloe vera is a plant different from other Crasulacean Acid Metabolsim (CAM) species because it is native of the semi tropical regions of South Africa (Cowling, 1982). The stomata of Aloe vera plants open during night and close during the day time and all exogenous gas exchange occurs at night. From the stomatal behaviour of IIHR $AV_{15}$ ie., the closure of stomata both during night and day (Fig. 2 and 3) the gaseous exchange and water loss from leaves were completely blocked led to watery blackened leaves.

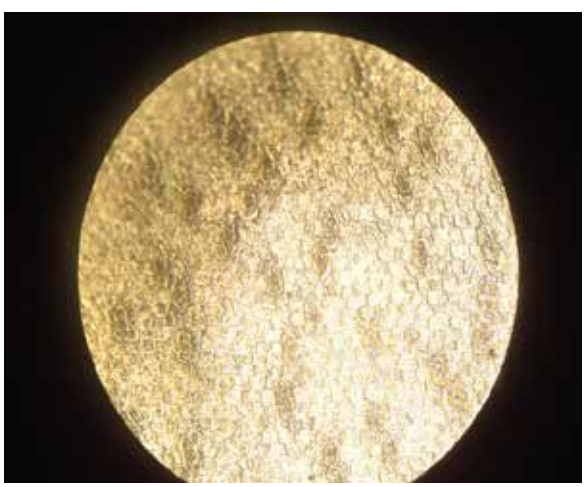

**Fig.2. Opened stomata in leaves of Aloe vera genotypes (except IIHR AV15) at night hours during waterlogging**

**Fig.3. Closed stomata in leaves of Aloe vera genotype IIHR AV15 at day and night hours due to waterlogging**

Oxygen deficiency generally leads to the substantial decline on net photosynthetic rate and this decrease in transpiration and photosynthesis is attributed to stomatal closure (Ashraf et al., 2011). The fast stomatal closure when subjected to waterlogging was also reported in Barley (Yordanova et al., 2005) and Pea (Zang and Zang, 1994).

**Perturbation in chlorophyll fluorescence**

Chlorophylls are the green pigments responsible for trapping of light energy for conversion into chemical energy through photosynthesis. Waterlogging stress is also known to cause marked disturbance in different chlorophyll fluorescence attributes of plants. Chlorophyll fluorescence is an excellent physiological marker that determine the primary process involved in photosynthesis such as energy transfer due to excitation, absorption of light and photochemical reactions occurring in the Photosystem II (PS II) (Saleem et al., 2011). Aloe vera genotype, IIHR AV15 could experience the alteration in chlorophyll fluorescence as the leaves were blackened from tip downwards. If otherwise the plants not rescued in time and if waterlogging persist for prolonged period, a prominent decrease in maximum quantum efficiency (Fv/Fm) could be noticed.

**Root injuries and formation of adventitious roots**

In the present study the Aloe vera genotype IIHR $AV_{15}$ showed the root injuries such as death of feeder roots and easy peel-off of bark besides emitting foul odour. Further the formation of adventitious roots was also noticed. Formation of adventitious roots potentially replacing the basal roots is considered as one of the potential morphological adaptations exhibited by plants under waterlogging stress (Malik et al., 2001). Under normal condition, sufficient $O_2$ is present in the soil atmosphere for the proper growth and development of the roots and for their physiological activities. More chronic situations due to excessive moisture could cause major death of the feeder root system. This is exhibited by wilt - to- death above ground. The adventitious root development observed at or above the soil line is the strong evidence for excessive soil moisture. This is also in conformity with the findings of Malik et al., (2005) in wheat. Theses specialized roots helps on maintaining the continuous supply of water and mineral when the main root system fails to do so. Though IIHR $AV_{15}$ susceptible to waterlogging stress, the production of adventitious roots as an adaptive mechanism proved its tolerance to the stress.

**Oxidative damage induced by Reactive Oxygen Species (ROS)**

Reduction of oxygen by any means could result in the production of Reactive Oxygen Species (ROS) that affects several cellular metabolic processes of plants (Ashraf, 2009). ROS are free radicals possessing one or more unpaired electrons. This is not a stable configuration; therefore, the radicals react with other cellular molecules to produce more free radicals. Different cellular organelles such as mitochondria, chloroplasts and peroxisomes are considered as the sites of production of reactive oxygen species (Sairam and Srivastava, 2002). The lethal effect ROS includes superoxide ($O_2^{-1}$), hydrogen peroxide ($H_2O_2$) and hydroxyl readical (OH). These ROS are inducing damage to a number of cellular molecules and metabolites such as proteins, lipids, pigments, DNA etc. It was found that it could also affect the metabolic reactions of plants such as photosynthesis and efficiency of PS II.

However all the plants have the ability to detoxify the adverse effect of ROS by producing different types of antioxidants. The antioxidants include enzymatic anti-oxidants (ascorbate peroxidase, superoxide dismuitase, peroxidase catalase) and non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols and carptenoids).

**Effect of waterlogging on nutrient composition of plant parts**

Waterlogging reduces the endogenous levels of nutrient in different parts of plants. Oxygen deficiency in the root zone causes a marked decline in the selectivity of $K^+/Na^+$

Volume : 3 | Issue : 5 | May 2013 | ISSN - 2249-555X

uptake and hinders the transport of $K^+$ to the leaves. It has also been reported in the literature that hypoxic conditions cause decrease in the permeability of root membrane to $Na^+$ (Barrett-Lennard et al., 1999). Generally waterlogging causes an acute deficiency of essential nutrients such as nitrogen, phosphorous, potassium, magnesium and calcium.

**Changes in $p^H$ values of soil**

The $p^H$ values of flooded soil were estimated on second day of waterlogging and on 7th day. It shows a reduced value of 6.5 on 2nd day and it reached the normal value of 7.5 on 7th day after flooding. The initial decrease in pH might be due to the result of an accumulation of $CO_2$ and production of organic acids. The increase in $p^H$ is associated with the reduction process of the soil. Retardation of reduction process slows the $p^H$ increase (Morachan, 1986).

**Evolution of toxic elements under waterlogged condition**

Soils lacking in $O_2$ become the hotbeds of decomposition by anaerobic Bacteria and the products of their activities. Hydrogen sulphide, ethylene and ammonia, are toxic to the roots and inhibit their development and metabolic activity. The genotype IIHR $AV_{15}$ might be more susceptible to these compounds and could be one among the reasons for the death of leaves.

**Genetic variability for waterlogging tolerance**

In the present investigation, among the twenty one genotypes of Aloe vera exposed to waterlogging for more than 96 hours, only one genotype showed its susceptibility to waterlogging stress with the ability to revive. The remaining twenty types showed their tolerance against waterlogging. The exact cause or the mechanism responsible for this effect was not determined and we can only surmise that it was due to individual plant variability caused by both genetic and phenotypic differences or other differences associated with special variability within the facility. Plants under waterlogged conditions exhibits marked up and/or down-regulation of a number of genes. By investigating the induced expression of these genes in low oxygen environment, it is possible to identify certain gene products. Then these potential genes involved in conferring waterlogging tolerance can be isolated and introduced into the transgenic plants in order to identify their possible contribution in stress tolerance.

**Rescue of affected plants**

In order to rescue those plants of genotype IIHR $AV_{15}$ affected by waterlogging, they were carefully pulled out from the waterlogged soil and replanted in the mud pot containing FYM, sand, red soil and vermiculite (1:2:1:1) as pot mixture. By this way the plants were relived from suffocation and further damage to the leaves at inner whorl was prevented and the plants were rescued safely for further study.

**Conclusion**

It can be inferred from the aforesaid discussion that waterlogging stress causes a wide variety of physiological alterations in plant system and physiochemical properties of soil Aloe vera could be adjudged as waterlogging tolerant plant species however the variability was found among the genotypes in degree of tolerance. The specific adaptive mechanisms present in the Aloe vera plant system has to be further explored. A deep genetic study is needed to identify the genes responsible for tolerance to waterlogging stress so that the potential genes involved in waterlogging tolerance can be isolated and introduced into the transgenic plants to reap maximum productivity. Except IIHR $AV_{15}$ all other genotypes studied could be potentially used for future breeding programmes.

**REFERENCE**

[1]. Anjos e Silva, S.D., Sereno MJC de M, Lemons e Silva CF and Barbosa Neto, J.F, (2005), Genetic parameters and QTL for tolerance of flooded soils in maize, Crop Breed.Appl.Biotechnol., 5, 287-293. | [2]. Ashraf, M., (2009), Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotech.Adv., 27, 84-93. | [3]. Ashraf, M.A., Ahmad, M.S.A, Ashraf, M and Ashraf, M.Y, (2011), Alleviation of waterlogging stress in upland cotton by exogenous application of potassium in soils and as a foliar spray, Crop Pasture Sci., 62(1), 25-38. | [4]. Barrett-Lennard, E.G., Ratingen, P.V. and Mathie, C.A., (1999), The developing pattern pf damage in wheat due to the combined stresses of salinity and hypoxia: Experiments under controlled conditions suggest a methodology for plant selection. Aust.J.Agr.Res., 50, 129-136. | [5]. Cowling, R.M. (1982), Patterns of plant endemism in the south east Cape. The Naturalist (27), 17-36. | [6]. Folzer, H., Dat, J., Badot, P.M., (2006), Response to flooding of sessile oak: An integrative study, Tree Physiol., 26, 759-766. | [7]. Gambrell, R.P. and Patrick, W.H., (1978), Chemical and microbiological properties of anaerobic soils and sediments. In: Plant Life in Anaerobic Environments, pp. 375-423. (Hook, D.D and Crawford, R.M.M., Eds.), Ann Arbor Sci.Publ., Ann Arbor, MI, U.S.A. | Gardner, W.K. and Flood, R.G, (1993), Less waterlogging damage with long season wheats. Cereals Res.Commun., 21(4), 337-343. | [8]. Malik Al, Colmer, T.D, Lamber, H and Schortemeyer, M, (2001). Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. Aust.J. Plant Physiol., 28, 1121-1131. | [9]. Malik Al, D. Colmer Trimothy, Lambers Hams and Schortemeyer Marcus, (2005), Changes in physiological and morphological traits of root and shoots of Wheat in response to different depths of waterlogging. Australian J. of Plant Physiol., 28(11), 1121-1131 | [10]. Morachan, Y.B. 1986. Crop production and management. Oxford & IBH Publishing Co., New Delhi, 219-246p. | [11]. Sairam, R.K., and Srivastava, G.C., (2002), Changes in antioxidant activity in sub-cellular fraction of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci., 162, 897-904. | [12]. Saleem, M., Ashraf, M and Akram, N.A., (2011), Salt (Nacl) induced modulation in some key physio-biochemical attributes in okra (Abelmoschus esculentus L.), J. Agron. Crop Sci., 197, 202-213. | [13]. Vartapetian, B.B, (1991), Flood-sensitive plants under primary and secondary anoxia: Ultra structural and metabolic responses. In: Plant Life under Oxygen deprivation, pp.201-206 (Jackson, M.B., Davies, D.D., and Lambers H., Eds.), SPB Academic Publ. Hague, Netherlands. | [14]. Yogeeswaran ,G., Anbarasu , S and Karthick. S. N (2005), Aloe vera : A miracle herb. Herbal tech Industry, 1 (8), 17-22 | [15]. Yordanova, R.Y., Uzunova, A.N and Popava, L.P, (2005), Effects of short-term soil flooding on stomatat behaviour and leaf gas exchange in Barley plants, Biolgia Plantarum, 49(2), 317-319. | [16]. Zang, J., and Zang, X, (1994). Can early wilting of old leaves account for much of the ABA accumulation in flooded pea plants, J.Exp. Bot., 45, 1335-1342. |