BIOLOGIA PLANTARUM 49 (3): 459-462, 2005

BRIEF COMMUNICATION

# Gas exchange characteristics and water relations in two cultivars of *Hibiscus esculentus* under waterlogging

M. ASHRAF* and M. ARFAN

*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan*

## Abstract

Thirty-day-old plants of two okra cultivars, Sabzpari and Chinese-red, were subjected for 30 d to normal watering or continuous flooding. Continuous flooding did not cause any adverse effect on shoot fresh and dry biomass. Leaf water potential and pressure potential of both cultivars increased significantly due to waterlogging, but there was a slight increase in leaf osmotic potential. Chlorophyll *a* and *b* contents decreased significantly and chlorophyll *a*/*b* ratio increased. Waterlogging caused a significant reduction in net photosynthetic rate, water use efficiency and intrinsic water use efficiency, but stomatal conductance and intercellular $CO_2$/ambient $CO_2$ ratio remained unchanged.

*Additional key words*: anaerobiosis, chlorophyll, flooding, net photosynthetic rate, okra, stomatal conductance, transpiration rate.

In waterlogged soils, water replaces oxygen in soil macro- and micro-pores and depletes oxygen thereby causing hypoxic conditions to the root system. In view of Liao and Lin (2001) tolerance to waterlogging, generally, is the ability of a plant to survive and continue growing after several days in submerged conditions. It is now evident that plants tolerant to waterlogging are capable of leaking oxygen from their root surfaces and oxidizing the reduced forms of iron and manganese thereby preventing the plants from taking up high amounts of these toxic ions (Bartlett 1961, Talbot *et al*. 1987, Ashraf and Yasmin 1991). Another means plants use to alleviate the stress is that they develop aerenchyma, which facilitates air conductance from shoots to roots (Armstrong 1982).

It has been reported that flooding causes a significant reduction in photosynthetic capacity particularly in most waterlogging-intolerant plants, *e.g.*, *Triticum aestivum* (Trought and Drew 1980), *Pisum sativum* (Jackson and Kowalewska 1983, Zhang and Davies 1987), *Lycopersicon esculentum* (Bradford 1983, Jackson 1990), *Lolium perenne* (McFarlane *et al*. 2003). In contrast, some waterlogging-tolerant plants have developed adaptive mechanisms so as to maintain better photosynthetic capacity (Sena Gomes and Kozlowski 1980, Li *et al*. 2004).

Okra (*Hibiscus esculentus* L.) is one of the important horticultural crops in many countries (Ashraf *et al.* 2003). Heavy rains during the season in some lowland areas cause waterlogging, which affect crop growth. However, little work has been done with this species with regard to its response to waterlogging. Thus the principal objective of this study was to assess the crop response to waterlogging by examining its growth, water relations, and gas exchange characteristics.

Seeds of two okra (*Hibiscus esculentus* L.) cultivars, Sabzpari and Chinese-red, were obtained from the Director Vegetables, Ayub Agricultural Research Institute, Faisalabad, Pakistan. The seeds were surface sterilized in 5 % sodium hypochlorite solution for 10 min. The experiment was conducted in a naturally-lit glasshouse in the Botanic Garden of the University of Agriculture, Faisalabad, Pakistan (31°30′ N, 73°10′ E and altitude 213 m), where the average PAR measured at noon ranged from 972 to 1776 μmol $m^{-2}$ $s^{-1}$, day/night relative humidity 28/54 % and temperature 44/31 °C. In April 2000, 15 seeds of each cultivar were sown randomly about 5 mm deep in earthen pots, which contained 10 kg sandy loam soil (pH = 7.76, electrical conductivity = 1.40 dS $m^{-1}$). After the emergence the

---

*Received* 18 August 2004, *accepted* 11 January 2005.

*Abbreviations*: $c_a$ - ambient $CO_2$ concentration; $c_i$ - intercellular $CO_2$ concentration; Chl - chlorophyll; E - transpiration rate; $g_s$ - stomatal conductance; $P_N$ - net photosynthetic rate.

* Corresponding author; fax: (+92) 41 9200764, e-mail: ashrafm@fsd.paknet.com.pk

plants were thinned to six in each pot. All the pots were irrigated for 30 d with normal irrigation water after which time flooding treatment was started by maintaining the water level in the treated pots up to the soil surface, whereas the control (unflooded) pots were watered normally. The moisture levels were monitored daily, whereas $O_2$ contents determined weekly with a *Dissolved Oxygen Meter Oxi 330i* (*WTW*, Weilheim, Germany). The experiment was arranged in a completely randomized design with 5 replicates.

30 d after the start of waterlogging a fully expanded youngest leaf (second from the top) was excised from each plant at 08:00 and the leaf water potential was measured with a Scholander type pressure chamber (*Arimad-2*, *ELE International*, Tokyo, Japan). A proportion of the leaf used for water potential determination was frozen for two weeks, thawed and the frozen sap was extracted by crushing the material with a metal rod. After centrifugation (8000 *g*) for 4 min, the sap was used for osmotic potential determination in a freezing point depression osmometer (*Osmolette-S 4002*, *Precision System Inc.*, Natick, USA).The difference between leaf osmotic potential and water potential was considered as pressure potential. Chlorophyll content was determined following the method described by Arnon (1949). The absorbances of the 80 % acetone extracts of leaf tissue were measured at 645, 652, and 663 nm using a spectrophotometer *Hitachi U-2000* (Tokyo, Japan). Measurements of net photosynthetic rate ($P_N$), transpiration rate (E) and stomatal conductance ($g_s$) were made on fully expanded youngest leaf of each plant (second leaf from top) using *LCA-4* portable photosynthetic system (*Analytical Development Company*, Hoddesdon, UK). Measurements were performed from 09:00 to 11:00 (PAR at leaf surface was maximum up to 1276 $\mu$mol $m^{-2}$ $s^{-1}$, temperature of leaf was maximum up to 40.9 °C, ambient temperature ranged from 32.2 to 38.2 °C, ambient $CO_2$ concentration 352.1 $\mu$mol $mol^{-1}$). After all these measurements, the plants were harvested. Plant roots were removed carefully from the soil and then were washed for 2 - 3 min in distilled water. Plants were separated into shoots and roots. Fresh masses of shoots and roots of all the plant samples were recorded. Samples were then dried at 65 °C for one week and dry masses recorded.

Data for all the parameters were subjected to a two-way analysis of variance using *COSTAT* computer package (*CoHort Software*, Berkeley, USA). The least significant differences between the mean values were calculated following Snedecor and Cochran (1980).

Waterlogging imposed for a period of 30 d caused a significant reduction in soil oxygen content (waterlogged 0.62 mg $dm^{-3}$; normally watered, 6.45 mg $dm^{-3}$). Waterlogging did not cause a significant reduction to fresh and dry mass of shoots in both cultivars, although shoot length and plant leaf area were reduced (Table 1). This shows that these cultivars are highly tolerant to waterlogged conditions, but up to what extent they are tolerant to long-term waterlogging, further experimentation is required to be carried out.

Waterlogging decreased $P_N$, water use efficiency ($P_N$/E), and intrinsic water use efficiency ($P_N/g_s$) of both okra cultivars (Table 1), but in contrast, there was no significant effect on $g_s$, and intercellular $CO_2$/ambient $CO_2$ concentration ($c_i/c_a$) ratio. Cultivars differed significantly in response of E to waterlogging:. E in Sabzpari decreased, whereas in Chinese-red it increased (Table 1). Although $P_N$/E and $P_N/g_s$ decreased considerably in both cultivars, the decrease in $P_N$/E was more marked in Chinese-red than that in Sabzpari, but the reverse was true for $P_N/g_s$ (Table 1).

In view of some reports stomatal regulation governs $CO_2$ exchange rate in plants subjected to waterlogging (Regehr *et al*. 1975, Phung and Knipling 1976). For example, a decrease in $g_s$ caused a reduction in $P_N$ due to lowering $c_i$ in flooded *Vaccinium ashei* (Davies and Flore 1986a,b), and *Fragaria* × *ananassa* (Blanke and Cooke 2004). However, $P_N$ and $g_s$ in two cultivars of okra do not show a significant relationship because $P_N$ decreased but $g_s$ and $c_i$ remained unaffected under waterlogged conditions. Similar results have been reported in sunflower (Guy and Wample 1984). Substomatal $CO_2$ concentration has also been found to be unaffected in flooded plants of pecan (Smith and Ager 1988), sweet orange grafted trees (Vu and Yelenosky 1991) and bitter melon (Liao and Lin 1996). Thus it is possible that in okra non-stomatal factors are responsible for reduction in $P_N$.

Waterlogging decreased $P_N$ but did not cause reduction in shoot biomass of both cultivars in agreement with the previous results with *Hibiscus cannabinus* (Curtis and Läuchli 1986), *Trifolium repens* (Rogers and Noble 1992) and *Triticum aestivum* (Hawkins and Lewis 1993, Ashraf and O’Leary 1996).

Stomatal regulation is known to govern water potential in plants subjected to flooding (Liao and Lin 1996). It has been observed in bitter melon by Liao and Lin (1994) that waterlogging caused reduction in $g_s$ thereby increasing leaf water potential. Conversely, in our study $g_s$ in both okra cultivars remained almost unchanged under waterlogged conditions, but leaf water potential increased considerably in flooded plants (Table 1). Thus increase in leaf water potential under flooding was found to be independent of stomatal control. In fact, osmotic potential and pressure potential are the main determinants of water potential (Taiz and Zeiger 2002). In the present study, there was a slight increase in leaf osmotic potential in flooded plants of both okra cultivars, but there was a marked increase in leaf pressure potential.

Waterlogging significantly reduced contents of both chlorophylls (Chl) *a* and *b*, but the reduction was more pronounced in Chl *b* than that in Chl *a* (Table 1). The cultivars did not differ significantly in both pigments. Chl *a/b* ratio increased significantly in both cultivars under waterlogged conditions (Table 1). Decline in Chl *b* under waterlogged conditions may have significant effect on LHC 2 complexes containing almost chlorophyll *b* in the mature thylakoid membranes (Green 1988).

Since the conclusions drawn in the present study are based on only two cultivars, further work is required to be carried out involving a large number of cultivars of okra to affirm such negative or positive relationships between growth and different physiological attributes.

Table 1. Different growth, water relations, and gas exchange attributes of two *Hibiscus esculentus* cultivars when 30-d-old plants were subjected for 30 d to waterlogged conditions. The attributes with no letters had interaction (waterlogging treatment × cultivars) non-significant. Means with the same letters within each column and each attribute do not differ significantly at $P = 0.05$ ($n = 5$).

| Parameter | Cultivars | Unflooded | Flooded |
|---|---|---|---|
| Shoot fresh mass [g plant$^{-1}$] | Sabzpari | 21.14 ± 1.94 | 21.10 ± 1.44 |
| | Chinese-red | 24.60 ± 2.83 | 23.02 ± 1.04 |
| Shoot dry mass [g plant$^{-1}$] | Sabzpari | 5.11 ± 0.47 | 4.46 ± 0.14 |
| | Chinese-red | 5.24 ± 0.51 | 5.25 ± 0.15 |
| Shoot length [cm] | Sabzpari | 52.00 ± 1.73 | 34.76 ± 3.06 |
| | Chinese-red | 57.50 ± 0.93 | 46.92 ± 2.26 |
| Leaf area [cm$^2$ plant$^{-1}$] | Sabzpari | 250.80 ± 32.8 | 234.39 ± 11.4 |
| | Chinese-red | 364.15 ± 55.2 | 215.66 ± 13.8 |
| Leaf water potential [-MPa] | Sabzpari | 0.98 ± 0.02 | 0.57 ± 0.01 |
| | Chinese-red | 1.14 ± 0.06 | 0.78 ± 0.09 |
| Leaf osmotic potential [-MPa] | Sabzpari | 1.08 ± 0.05 | 0.95 ± 0.02 |
| | Chinese-red | 1.32 ± 0.05 | 1.09 ± 0.02 |
| Leaf pressure potential [MPa] | Sabzpari | 0.16 ± 0.03a | 0.55 ± 0.01a |
| | Chinese-red | 0.21 ± 0.03a | 0.47 ± 0.04a |
| Chlorophyll *a* [mg g$^{-1}$(d.m.)] | Sabzpari | 3.41 ± 0.03 | 2.44 ± 0.23 |
| | Chinese-red | 3.87 ± 0.02 | 2.58 ± 0.14 |
| Chlorophyll *b* [mg g$^{-1}$(d.m.)] | Sabzpari | 1.84 ± 0.09 | 0.78 ± 0.76 |
| | Chinese-red | 2.06 ± 1.08 | 0.91 ± 0.79 |
| Chlorophyll *a*/*b* ratio | Sabzpari | 1.86 ± 0.76 | 3.11 ± 0.04 |
| | Chinese-red | 1.88 ± 0.10 | 2.84 ± 0.11 |
| Net photosynthetic rate [µmol($CO_2$) m$^{-2}$s$^{-1}$] | Sabzpari | 13.91 ± 0.84 | 7.18 ± 0.97 |
| | Chinese-red | 10.91 ± 0.49 | 7.52 ± 1.07 |
| Transpiration rate [mmol($H_2O$) m$^{-2}$ s$^{-1}$] | Sabzpari | 6.13 ± 0.26a | 5.05 ± 0.61 a |
| | Chinese-red | 3.07 ± 0.20b | 5.55 ± 0.51 a |
| $P_N$/E [µmol($CO_2$) mmol$^{-1}$($H_2O$)] | Sabzpari | 2.31 ± 0.20a | 1.68 ± 0.20 a |
| | Chinese-red | 2.97 ± 0.19b | 1.36 ± 0.16 a |
| Stomatal conductance [mmol m$^{-2}$ s$^{-1}$] | Sabzpari | 281.40 ± 6.25 | 276.00 ± 18.0 |
| | Chinese-red | 345.00 ± 25.6 | 290.00 ± 43.4 |
| $P_N/g_s$ [µmol($CO_2$) mmol$^{-1}$($H_2O$)] | Sabzpari | 0.05 ± 0.003a | 0.02 ± 0.004 a |
| | Chinese-red | 0.03 ± 0.001b | 0.02 ± 0.003 a |
| $c_i/c_a$ ratio | Sabzpari | 0.77 ± 0.02 | 0.76 ± 0.036 |
| | Chinese-red | 0.74 ± 0.02 | 0.78 ± 0.26 |

## References

Armstrong, W.: Waterlogged soils. - In: Etherington, J.R. (ed.): Environment and Plant Ecology. Pp. 290-330. Wiley, Chichester 1982.

Arnon, D.T.: Copper enzyme in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Ashraf, M., Arfan, M., Ahmad, A.: Salt tolerance in okra: ion relations and gas exchange characteristics. - J. Plant Nutr. **26**: 63-79, 2003.

Ashraf, M., O'Leary, J.W.: Responses of some newly developed salt-tolerant genotypes of spring wheat to salt stress: II. Water relations and photosynthetic capacity. - Acta bot. neerl. **45**: 29-39, 1996.

Ashraf, M., Yasmin, H.: Differential waterlogging tolerance in three grasses of contrasting habitats: *Aeluropus lagopoides* (L.) Trin., *Cynodon dactylon* (L.) Pers. and *Leptochloa fusca* (L.) Kunth. - Environ. exp. Bot. **31**: 437-445, 1991.

Bartlett, R.J.: Iron oxidation proximate to plant roots. - Soil Sci. **92**: 372-379, 1961.

Blanke, M.M., Cooke, D.T.: Effect of flooding and drought on stomatal activity, transpiration, photosynthesis, water potential and water channel activity in strawberry stolons and leaves. - Plant Growth Regul. **42**: 153-160, 2004.

Bradford, K.I.: Effects of soil flooding on leaf gas exchange of tomato plants. - Plant Physiol. **73**: 475-479, 1983.

Curtis, P.S., Läuchli, A.: The role of leaf area development and photosynthetic capacity in determining growth of kenaf under moderate salt stress. - Aust. J. Plant Physiol. **13**: 353-

365, 1986.

Davies, F.S., Flore, J.A.: Short-term flooding effects on gas exchange and quantum yield of rabbiteye blueberry (*Vaccinium ashei* Reade). - Plant Physiol. **81**: 289-292, 1986a.

Davies, F.S., Flore, J.A.: Gas exchange and flooding stress effects of highbush and rabbiteye blueberries.- J. amer. Soc. hort. Sci. **111**: 565-571, 1986b.

Green, B.R.: The chlorophyll-protein complexes of higher plant photosynthetic membranes. - Photosynth. Res. **15**: 3-32, 1988.

Guy, R.D., Wample, R.L.: Stable carbon isotope ratios of flooded and unflooded sunflower (*Helianthus annuus*). - Can. J. Bot. **62**: 170-1774, 1984.

Hawkins, H.J., Lewis, O.A.M.: Combination effect of NaCl salinity, nitrogen form and calcium concentration on the growth and ionic content and gaseous properties of *Triticum aestivum* L. cv. Gamtoos. - New Phytol. **124**: 161-170, 1993.

Jackson, M.B.: Hormones and developmental change in plants subjected to submergence or soil waterlogging. - Aquatic Bot. **38**: 49-72, 1990.

Jackson, M.B., Kowalewska, A.K.B.: Positive and negative messages from roots induce foliar desiccation and stomatal closure in flooded pea plants. - J. exp. Bot. **34**: 493-506, 1983.

Li, S.W., Pezeshki, S.R., Goodwin, S.: Effects of soil moisture regimes on photosynthesis and growth in cattail (*Typha latifolia*). - Acta oecol. **25**: 17-22, 2004.

Liao, C.T., Lin, C.H.: Effect of flooding stress on photosynthetic activities of *Momordica charantia*. - Plant Physiol. Biochem. **32**: 1-5, 1994.

Liao, C.T., Lin, C.H.: Photosynthetic responses of grafted bitter melon seedlings to flooding stress. - Environ. exp. Bot. **36**: 167-172, 1996.

Liao, C.T., Lin, C.H.: Physiological adaptation of crop plants to flooding stress. - Proc. nat. Sci. Counc. ROC(B) **25**: 148-157, 2001.

McFarlane, N.M., Ciavarella, T.A., Smith, K.: The effect of waterlogging on growth, photosynthesis and biomass allocation in perennial ryegrass (*Lolium perenne* L.) genotypes with contrasting root development. - J. agr. Sci. **141**: 241-248, 2003.

Phung, H.T., Knipling, E.B.: Photosynthesis and transpiration of *Citrus* seedlings under flooded conditions. - Hort. Sci. **11**: 131-133, 1976.

Regehr, D.L., Bazzaz, F.A., Boggess, W.R.: Photosynthesis, transpiration and leaf conductance of *Populus deltoides* in relation to flooding and drought. - Photosynthetica **9**: 52-61, 1975.

Rogers, M.E., Noble, C.L.: Variation in growth and ion accumulation between two selected populations of *Trifolium repens* L. differing in salt tolerance. - Plant Soil. **146**: 131-136, 1992.

Sena Gomes, A.R., Kozlowski, T.T.: Growth responses and adaptations of *Fraxinus pennsylvanica* seedlings to flooding. - Plant Physiol. **66**: 267-271, 1980.

Smith, M.W., Ager, P.L.: Effect of soil flooding on leaf gas exchange of seedling pecan trees. - HortScience **23**: 370-372, 1988.

Snedecor, G.W., Cochran, W.G.: Statistical Methods. 7th Edition. - The Iowa State University Press, Ames 1980.

Taiz, L., Zeiger, E.: Plant Physiology. 3rd edition. - Sinauer Associates Inc. Publishers, Sunderland 2002.

Talbot, R.J., Etherington, J.R., Bryani, J.A.: Comparative studies of plant growth and distribution in relation to waterlogging. XII. Growth, photosynthetic capacity and metal ion uptake in *Salix caprea* and *S. cinerea* spp. *Oleifolia*. - New Phytol. **105**: 563-574, 1987.

Trought, M.C.T., Drew, M.C.: The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.). I. Shoot and root growth in relation to changes in the concentration of dissolved gases and solutes in the soil solution. - Plant Soil. **54**: 77-94, 1980.

Vu, C.V., Yelenosky, G.: Photosynthetic responses of citrus trees to soil flooding. - Physiol. Plant. **13**: 669-688, 1991.

Zhang, J., Davies, W.J.: ABA in roots and leaves of flooded pea plants. - J. exp. Bot. **38**: 649-659, 1987.