Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 8, 2015, pp. 3238-3245
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION FOR DROUGHT TOLERANCE IN 20 VIRGINIA GROUNDNUTS (*Arachis hypogaea* L.) CULTIVARS

Jinesh Kaneriya and Sujit Bishi

Department of Biochemistry Shree H.N.Shukal College of Science Rajkot
B/H Marketing Yard, Near LalPari Lack,Aamargadh-Bhichari,Rajkot.

Abstract

The research was performed to study the effect of water deficit on growth and physiological, biochemical constituents of twenty Virginia varieties of groundnut (*Arachis hypogaea* L.) Somnath, BAU, LGN 2 ,ICGV 8632,M 13 ,HNG 10, DRG 17, ICGS 76, ICGS 5 ,DSG 12,GG 14 GAUG 10 ,CSMG 84-1, Girnar 2 ,CSMG 884 ,GG 11 GG 20 ,Kadiri 3 ,B 95 ,GG 16. Gujarat is among the top groundnut producing states in India. Under rain-dependent cultivation of groundnut drought is a main constraint to increase the productivity, however, Virginia type are considered to be more drought tolerant than the Spanish type. Analysis of past 20 years rainfall data showed that in Gujarat end-of- season drought is a common phenomenon and Virginia type being having long crop duration are prone to end-of-season drought. Physiological parameters such as specific leaf area (SLA) an indicator of water use efficiency, relative water content (RWC), harvest index and total biomass, and biochemical parameter such as total free amino acids, proline, reducing sugars, total phenols, and methanol soluble and insoluble proteins in different plant parts i.e. leaf, stem, root, and seed after final harvest. Result showed that accumulation of total amino acid, and proline, under water deficit increased maximum in stem while reducing sugars, total phenols content and methanol soluble proteins increases in root parts and methanol insoluble proteins in stem under water-deficit condition.

Key words: Drought stress, Phenol, reducing sugar, phenol, Amino acid, SLA specific leaf area, RWC relative water content.

INTRODUCTION

Groundnut (Arachis hypogaea L) is a native of South American, is an important legume, cash crop, cultivated for several decades. Evidence showed that groundnut was domesticated in prehistoric times in Peru. Virginia market type groundnut is either bunch or runner in growth habit. The bunch type is upright to spreading. Groundnut responses to drought by increasing leaf diffusive resistance, reducing transpiration and folding of leaflet and their orientation to the sun light reduces the radiation load resulting to maintain the transpiration and leaf temperature.

In case of drought various physiological parameters i.e. stomata conduct ants, transpiration, photosynthesis, leaf relative water content (RWC) and leaf water potential are affected adversely. The root length, shoot length, total leaf area, plant fresh weight and dry weight are reduced significantly under water-deficit conditions.

The wild Arachis species are considered more drought and heat tolerant than the cultivated groundnut and their leaves are characterized by the presence of thick epicuticular wax, which intern helps in the maintenance of stomata conductance and water loss from the leaf, and may contribute to adaptation to the environments characterized by drought and heat stress [1].
Under sever water stress conditions plants stop growing completely and accumulate solutes in cells to maintain the cell volume against dehydration. This phenomenon is known as osmotic adjustment. Osmotic adjustment has been observed in leaf, stem, root and seed in groundnut plant [2];[3]. Low water potential induces a change in plants metabolism from its normal pattern, for example, total soluble sugar increases under water-deficit stress, enhances proteolysis, depress protein synthesis a de -novo enhance synthesis of amino acids, incorporation of proteins.
Thus the compatible solutes may be classified into two categories: one is nitrogen-containing compounds such as proline and other amino acids, quaternary ammonium compounds (QAC), glycinebetaine (GB) and polyamines, and the other are hydroxyl compounds, such as sucrose, polyhydric alcohols and oligosaccharides. Accumulation of amino acid, proline, and sugar in several plant species is regarded as general responses to drought stress in addition to this some amino acid (betain, glutamate, glutamine, asparagines) were found in higher tissues [4]. The accumulation of proline is indicator of drought stress and soluble sugar content proves to be batter marker for selective improvement of drought tolerance.
Accumulation of sugar was reported higher in root and stem under water-deficit as compared to unstressed plant [5]. Accumulation of mannitol, sorbitol, reffinose, trehalose, sucrose, these compounds help the cell to maintain their hydrated state and provide resistance against drought stress and cellular dehydration ([5],[6]

MATERIAL AND METHODS

Field layout

Twenty groundnut cultivars belonging to Virginia market type were sown in three time replicated traits in a Randomized Complete Design (RCBD) in the field under rain dependent and protected irrigation conditions, during rainy season (2009). The seed-to-seed distance was 10 cm and row-to-row distance was 45 cm and depth of sowing was 5 cm. Thus the treatments were protected irrigation (T1) and rain-dependent (T2) conditions.

Growth measurements

The number of flowers was recorded on five randomly selected plants from each plot and treatment from the date of first flower until 82 d after sowing. Specific leaf area (SLA), the ratio of fresh leaf area to leaf dry weight was calculated by measuring leaf area by leaf area meter (model: LI 3000). Total biomass was measured Pod weight recorded at final harvest at moisture content between 7 and 8%. At the final harvest, the number of mature and immature pods, pod yield and the harvesting index was calculated; Harvest index was calculated the ratio of total pod weight at the final harvest to the total biomass at the final harvest.

Plant sampling for biochemical analysis.

After 25 days of drought spell plants were up rooted and separated into different parts i.e. leaf, stem and root. Plant samples i.e. leaf (1.5 g), stem (5 g) and root (2.5 g) were killed in 80% methanol for 2 minutes and stored at 170C until use. After one month plant tissues were extracted in 20 ml of 80% boiling methanol on a hot plate for 1h. Supernant was collected and filtered through whatman filter paper No.1. In the remaining tissue another 20 ml of 80% methanol was added and the extraction procedure was repeated. After completing the extraction final volume was made up to 50 ml with 80% methanol. The extract was used for the determination of free proline, reducing sugar, total phenol, total free amino acids and methanol soluble proteins, whereas residue was used for determination of methanol insoluble proteins.

Biochemical Parameters

Estimation of Proline

For estimation of free proline the method described by Bates *et al.* (1973) was followed.Standard curve was prepared by using different concentration of proline, ranging

between 2 µg and 10 µg. After developing the colour concentration of the proline was read at 520 nm with the help of a spectrophotometer. For estimating free proline in the samples, 0.2 ml of methanol extract was taken to make the final volume to 2 ml with distilled water. Two ml of glacial acetic acid was added followed by the addition of 2 ml acid ninhydrin reagent in the test tubes, replicated three times. The test tubes were placed in a water bath at 100°C for 1 h. After 1 h the test tubes were cooled down in an ice bath for terminating the reaction. After 10 minutes 4 ml of toluene was added and test tubes were shaken vigorously for 15-20 seconds, upper toluene layer was collected and OD was measured at 520 nm.

Estimation of Reducing Sugar

Reducing sugars were estimated by following the method described by Somogyi (1945). Standard curve was prepared by using different concentration of glucose solution ranging between 20 µg and 100 µg. After developing the colour concentration of the reducing sugar was read at 620 nm in a spectrophotometer. For estimating reducing sugars, 0.2 ml methanol extract was taken to make final volume to 2 ml with distilled water followed by addition of 2 ml copper reagent C in test tubes, replicated three times. The test tubes were placed on boiling water bath for 10 minutes. After 10 minutes test tubes was cooled and 1 ml Nelson's reagent was added. The test tubes were mixed gently and after 10 minutes colour concentration was measured at 620 nm in a spectrophotometer.

Estimation of Free amino acid

For estimating total free amino acid the method described by Moore and stein (1948) was followed. Standard curve was prepared by using different concentration of amino acids (Glycine, Lucine, Valine, Arginine, and Alanine) solution ranging between 10 µg and 50 µg. After developing the colour the reading was taken read at 570 nm with a spectrophotometer. For estimation of the total free amino acids in plant samples, 0.2 ml methanol extract was taken and 1ml ninhydrin was add to the solution and make the final volume was made up to 2 ml with distilled water. The test tubes were placed in boiling water bath for 20 minutes. After 20 minutes test tubes were cooled and 5 ml diluent solution was add after cooling, test tubes were shaken vigorously and after 15 minutes the O.D. was measured at 570 nm in a spectrophotometer.

Estimation of methanol soluble and insoluble

Methanol soluble and insoluble proteins were estimated using the method described by Lowry et al. (1951). Standard curve was prepared by using different concentration of BSA solution ranging between 40 µg and 200 µg. After developing the colour, read at 660 nm. For estimation of methanol soluble proteins 0.2 ml methanol extract was taken and used for protein estimation. However, for estimation of methanol insoluble proteins, 0.1 M NaOH was added to 100 mg methanol insoluble residues and kept at room temperature for overnight, Next day 0.2 ml supernatant was used for protein estimation. The volume in all the test tubes was made 1 ml with distilled water. After that 5 ml of solution C was added and properly mixed, after 10 minutes 0.5 ml of Folin's reagent (solution D) was added, mixed well all test tubes were kept at room temperature in dark for 30 minutes. After 30 minute concentration of colour was measured at 660 nm.

Estimation of total phenol

Total phenols were estimated using method described by Malick and Singh (1980). Standard curve was prepared by using different concentration of catechol solution ranging between 20 µg and 100 µg. After developing the colour concentration of the catechol was read at 650 nm. For estimation of total phenols in sample 0.2 ml methanol extract was taken to make up to 3 ml with distilled water, followed by the addition of 0.5 ml Foline-Ciocalteau reagent. After 3 min 2 ml of 20% sodium bicarbonate solution was added and test tubes were placed in boiling water bath for one minute. The test tubes were cooled and O.D. measured at 650 nm

*All the statistical analysis were done using Microsoft office excel 2013.

RESULT

Under water deficit condition the various biochemical parameters like proline, reducing sugar, total free amino acid, and total phenol Methanol soluble and insoluble proteins, are increase in various plant parts like leaf, stem, root, whole plants and seeds.

Among 20 Virginia cultivars under irrigated and water deficit condition shown Variety, Treatment, VXT, CV%. Under Irrigated and Water Deficit Condition.

	Variety	Tretment	VxT	CV(%)		Variety	Tretment	VxT	CV(%)
Free Proline					**Total Phenol**				
Leaf	0.02	0.01	0.02	2.84	Leaf	0.14	0.04	0.19	5.58
Stem	0.01	0	0.01	2.15	Stem	0.003	0.001	0.004	0.53
Root	0.01	0	0.01	1.21	Root	0.12	0.04	0.16	0.15
Seed	0.01	0	0.01	1.77	Seed	0.008	0.003	0.011	0.15
Reducing Sugar					**Methanol Solubal Protein**				
Leaf	0.2	0.06	0.28	0.88	Leaf	0.037	0.012	0.053	0.2
Stem	1.03	0.33	1.46	4.34	Stem	0.008	0.003	0.011	0.16
Root	0.354	0.112	0.501	7.29	Root	0.006	0.002	0.009	0.07
Seed	0.02	0.01	0.02	0.09	Seed	6.97	2.2	9.85	3.84
Total Free A.A					**Methanol In Solubal Protein**				
Leaf	0.06	0.019	0.085	1.38	Leaf	0.003	0.001	0.005	0.06
Stem	0.089	0.028	0.126	5.47	Stem	0.003	0.001	0.004	0.5
Root	0.073	0.023	0.104	1.43	Root	0.001	0.001	0.002	0.08
Seed	0.016	0.005	0.023	0.23	Seed	0.04	0.13	0.06	0.26

EFFECT OF WATER DEFICIT CONDITION ON VARIOUS BIOCHEMICAL PARAMETER

Here Represent change in different biochemical parameters shown a) leaf b) stem c) root d) whole plant; e) seed average value under irrigated and water-deficit condition in different plant parts (SE ± depected over the bars).(MSP=Methanol soluble Proteins) (MSIP= Methanol insoluble Protein.)

CORRELATIONS BETWEEN BIOCHEMICAL PARAMETERS AND YIELD ATTRIBUTES

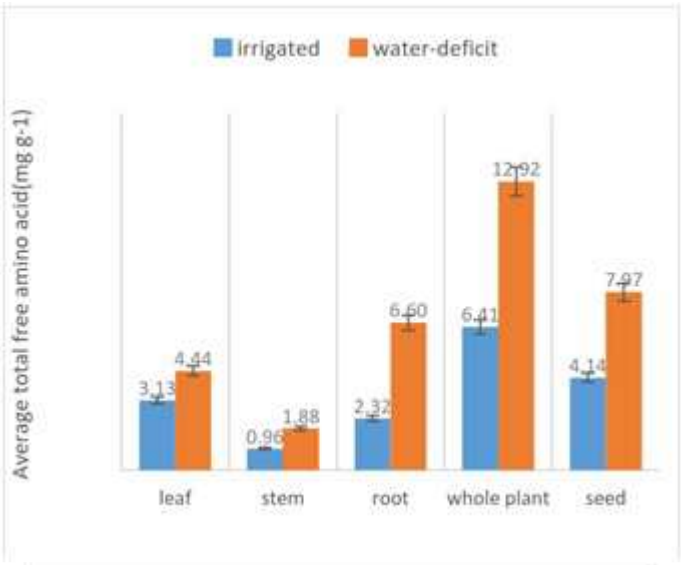
Among 20 Virginia cultivars, analysed under normal and water-deficit conditions during pod development stage. Various correlations were established between biochemical parameters and yield attributes.

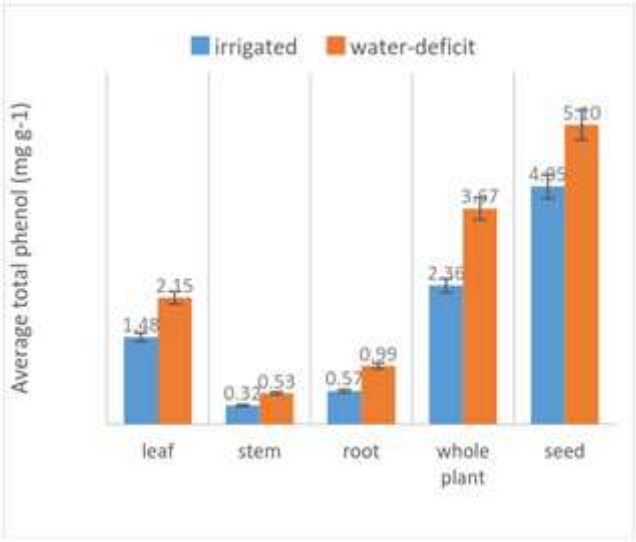
Under water deficit. Stem proline was directly associated with stem total amino acids (r=0.45*) and root total amino acids (r=0.91**) under water-deficit. Root proline was directly associated with leaf proline (r=0.39*) and stem proline (r=0.92**), under water-deficit.

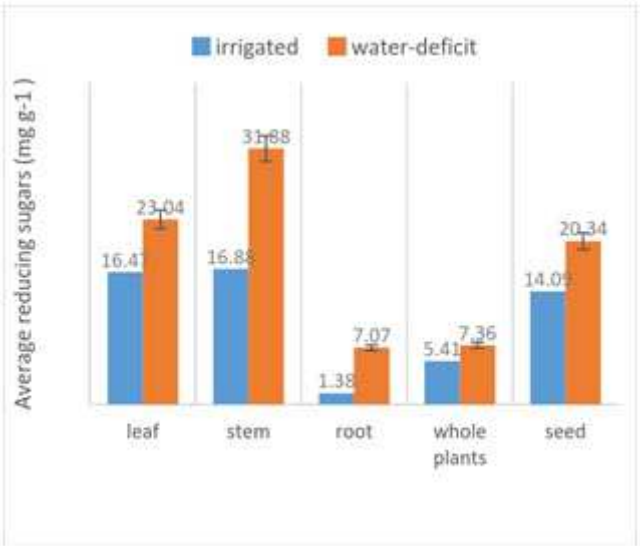
Root proline was directly also associated with total amino acid in leaf under irrigated (r=0.44*) and stem total amino acids (r=0.82**) and root total amino acids (r=0.62**) under water-deficit conditions.

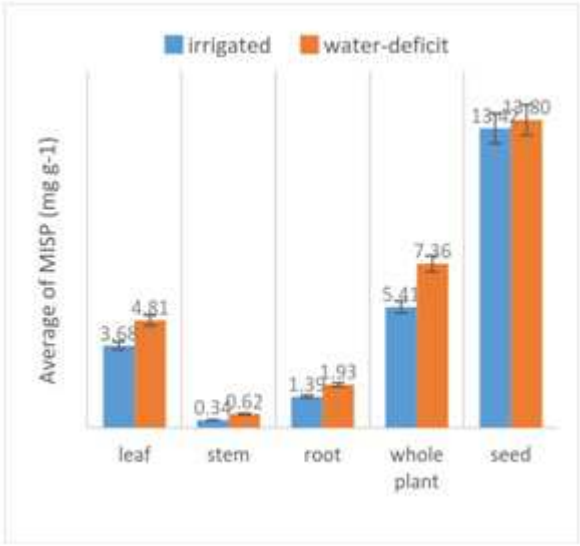
Various co-relations between the biochemical parameters and yield and yield attributes were worked out, among there harvesting index was directly associated with stem proline (r=0.56**). Harvest index was also associated with seed reducing sugar (r=0.41*), stem phenol (r=0.47*), under irrigated, and leaf phenol (r=0.47*) and leaf total amino acids (r=0.45*), under water-deficit conditions.

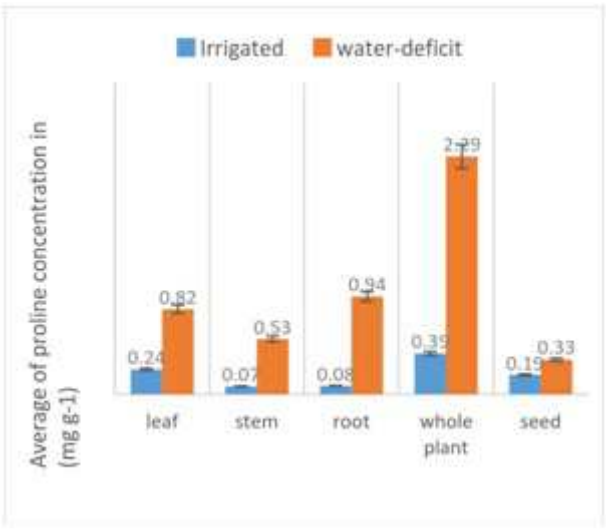
Pod yield was observed to be positively associated with harvest index (r=0.50**) Also there were positively association between stem phenols (r=0.48*) and root methanol soluble proteins (r=0.39*) and negatively association between stem methanol insoluble proteins (r=-0.48*) and pod yield, under irrigated condition.

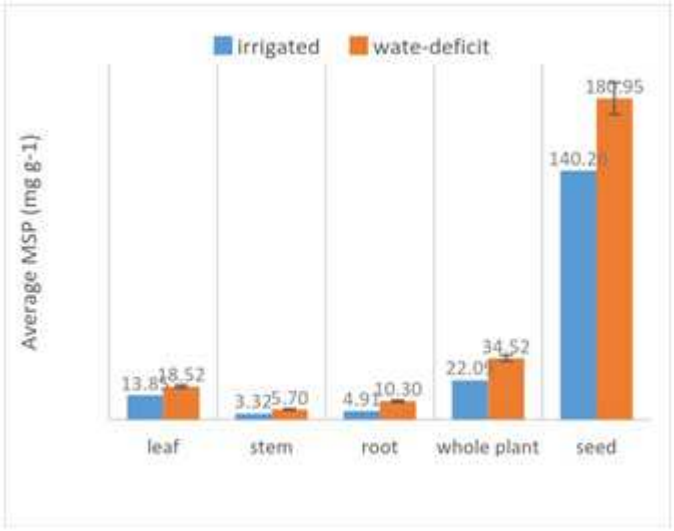

irrigated
water-deficit
Average total free amino acid(mg g-1)
3.13
4.44
0.96
1.88
2.32
6.60
6.41
12.92
4.14
7.97
leaf
stem
root
whole plant
seed


irrigated
water-deficit
Average total phenol (mg g-1)
1.48
2.15
0.32
0.53
0.57
0.99
2.36
3.67
4.09
5.10
leaf
stem
root
whole plant
seed


irrigated
water-deficit
Average reducing sugars (mg g-1)
16.41
23.04
16.88
31.68
1.38
7.07
5.41
7.36
14.09
20.34
leaf
stem
root
whole plants
seed







DISCUSSION

In groundnut Virginia type of groundnut cultivars are considered relatively drought tolerant, probably due to their closeness with the wild *Arachis* species. In addition to this the crop duration in this group is relatively longer than the Spanish type. Thus water-deficit stress in Virginia type is compensates to a great extent, if short spell of drought encountered during early stage of flowering and pod imitation[1] .

Thus accumulations of free amino acids are utilized by the plant to reduce the effects of the water-deficit through organic solute accumulation by increasing the water retention capacity[7].

Another important osmoprotectant is the sugars concentration therefore the distribution of sugars in different plant parts under normal and water-deficit conditions was investigated. It seems that the increase in the level of reducing sugars in plant is regulated by starch degradation pathway through amylase enzyme action and sugars, mannitol and alcohols in responses to osmotic stress is initiated [8].

In addition, phenols are secondary metabolites in plant and produced as a by-product of primary metabolism. Increased phenols in leaf under water-deficit, in this study, indicated that it may help plant to increase the intensity of the blue fluorescence originating from plant phenolic to protect leaf from high radiations [9],[10]

Reduction in methanol soluble proteins under water stress in this study could be ascribed to increase in proteases enzyme activity as proteases promote the breakdown of proteins and consequently decrease the protein amount presents in the plant under stress conditions [11],[12].

In Virginia type under water-deficit condition fresh vegetative growth triggers along with the new flowers and thus generating a competition between pod already developed and new leaves and flowers being produced. This advantage in Virginia groundnut could be exploited if the water deficit stress is bit early during flowering and pegging stage by delayed harvesting (Nautiyal P.C. unpublished data). Thus based on accumulation of proline in stem, phenols in leaf, RWC and HI a criteria was fixed to identify low and high parameter cultivars as:

Drought tolerant cultivars			Drought susceptible cultivars		
Stem proline	Leaf phenols	HI	Stem proline	Leaf phenols	HI
GG 16	M 13	GG 11	Girnar 2	B 95	HNG 10

CONCLUSION

In conclusion, we have identified drought tolerant groundnut cultivars based on the physiological and biochemical traits. Further, accumulation of proline in different plant parts showed that stem proline could be used as an indicator of drought tolerance. Reducing sugars accumulated in leaf and proline in stem, indicating their respective role in drought tolerance. Moreover, the change in MS and MIS proteins in seed may be used as a method to assess the quality as influenced by drought stress. Therefore to take advantage physiological and biochemical parameters for increasing productivity, we need to identify the drought tolerant and drought susceptible cultivars.

REFERENCES

1. Nautiyal, P., et al., *Evaluation of wild Arachis species for abiotic stress tolerance: I. Thermal stress and leaf water relations.* Euphytica, 2008. **159**(1-2): p. 43-57.
2. Nonami, H. and J.S. Boyer, *Turgor and growth at low water potentials.* Plant Physiology, 1989. **89**(3): p. 798-804.
3. Patakas, A., et al., *The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines.* Plant Science, 2002. **163**(2): p. 361-367.
4. Drossopoulos, J., A. Karamanos, and C. Niavis, *Changes in free amino compounds during the development of two wheat cultivars subjected to different degrees of water stress.* Annals of botany, 1985. **56**(3): p. 291-305.
5. Mohammadkhani, N. and R. Heidari, *Drought-induced accumulation of soluble sugars and proline in two maize varieties.* World Appl. Sci. J, 2008. **3**(3): p. 448-453.
6. Garg, A.K., et al., *Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses.* Proceedings of the National Academy of Sciences, 2002. **99**(25): p. 15898-15903.
7. Šircelj, H., et al., *Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought.* Journal of plant physiology, 2005. **162**(12): p. 1308-1318.

8. Chaves, M. and M. Oliveira, *Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture.* Journal of experimental botany, 2004. **55**(407): p. 2365-2384.
9. Cerovic, Z., et al., *The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves.* Plant, Cell & Environment, 2002. **25**(12): p. 1663-1676.
10. Spergel, D.N., et al., *First-year Wilkinson Microwave Anisotropy Probe (WMAP) observations: determination of cosmological parameters.* The Astrophysical Journal Supplement Series, 2003. **148**(1): p. 175.
11. Debouba, M., et al., *NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato "Lycopersicon esculentum" seedlings.* Journal of plant physiology, 2006. **163**(12): p. 1247-1258.
12. Ramos, A., et al., *AC electric-field-induced fluid flow in microelectrodes.* Journal of colloid and interface science, 1999. **217**(2): p. 420-422.