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The Altered Output of *Aloe vera* (L.) Burm. *f*. Crop under Differential Water Stress Conditions

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Abstract

Experiments were conducted the xerophytic *Aloe vera* (L.) Burm. *f*. plant samples of three age group *viz.* 20 days, 40 days and 60 days old plants in order to estimate the change in productivity (total yield) and protein content under differential water stress conditions. The plantlets were subjected to different water stress conditions under excess, optimum and deficient water supply under water-logging, daily-watering, alternate-day, bi-weekly, once-a-week water supply conditions. It was found that the total productivity and protein content was maximum in once-a-week watered plants and minimum in daily-watered plants indicating that the cultivation practice adopted for best production of the crop should be based on intermittent weekly rather than continuous water supply to the plants. Water-logged conditions were detrimental for their survival.

Keywords: *Aloe vera*, CAM pathway, xerophytes, productivity, protein content, water stress

1. Introduction

The present crisis originating from ever growing population results in an increased demand of food products, drugs, medicines, cosmetics and other health products. To meet the requirements an incessant influx of plant substances is essential for all pharmaceutical industries' establishments which are sustained on plant-based raw materials that cannot be synthesized by any artificial methods. This necessitates investigations on medicinal plants in terms of their productivity, useful proteins and other components which vary from species to species. In this background the xerophytic plant *Aloe vera* (L.) Burm. *f.* with super drought resistance and CO_2 reduction pathway of Crassulacean Acid Metabolism (CAM) is a highly useful plant which possesses special functions and high values in medicine, food industry, surfactant detergent and cosmetics, and health care due to its high bioactivity [1, 2, 3].

Morphologically, the plants are stem less with triangular, fleshy leaves having serrated edges, yellow tubular flowers and fruits with numerous seeds. The leaf epidermis is thick and covered with a cuticle surrounding the mesophyll, which is differentiated into outer layer of thick walled chlorenchymatous cells (skin) and inner thinner walled parenchyma (fillet). The parenchyma cells contain a transparent mucilaginous jelly which is known as *Aloe vera* gel [4, 5]. Chemically, the gel constitutes of anthraquinones including the hydroxyathracene derivatives, aloin A and B, barbaloin, isobarbaloin and aloe amedin [6].

The paramount value *Aloe vera* (L.) Burm. *f.* in medicine and cosmetic industry for it's wide scale applications in healing of wounds and as anti-inflammatory, antibiotic, anti-bacterial, anti-viral, anti-fungal, anti-diabetic and anti-neoplastic utility against some diseases (diabetes, cancer and allergy; [3, 7–10]). Due to the large scale economic importance, the production of *Aloe vera* is currently being adopted at an accelerated pace throughout the world [7]. Cultivation and crop production of *Aloe vera* is often exposed to unaccounted barriers resulting in an insufficient yield,

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low gel content and leaf contamination by pathogens and /or pests [11]. Being tropical in nature, the *Aloes* cannot withstand extremes of temperatures [12], which, adds on to the restricted production acreage. Thus, the cultivation of this species under semi-arid environment is not the same as other native species. When *Aloe vera* is grown under normal water conditions, it behaves as a typical CAM plant, but when exposed to stress condition of excess water supply, it shifts to CAM-idling (a dampened form of CAM), in which the stomata get closed day and night but with a continued, low diurnal organic acid fluctuation.

Earlier experiments on *Aloe vera* cultivation were focused on water stress conditions under depleted water supply only, which revealed a steady decrease in Relative Water Content (RWC), to the extent of *c.* 50%, at which, the water potential decreases precipitously and the Abscisic Acid (ABA) increases sharply. When re-watered, the metabolism quickly returns to the typical CAM pathway [13]. There is no change in chlorophyll, protein, and ribulose bisphosphate carboxylase activity in water-deficient plants compared with the well-watered plants. The carbohydrate content and anthraquinone glycosides also increased under water stress conditions in the *Aloe* plants [14]. Mustafa [14] and Tawfik [15] demonstrated that short intervals of irrigation of *Aloe* increased the chlorophyll content but reduced amino acid [16].

Understanding the requirement of effect of differential water stress conditions including the effect of excess water supply to *Aloe vera* plants on the total productivity and protein constitution the present experiment was conducted. The affect of differential water supply ranging from daily, Alternate-days, bi-weekly, Once-a-week and water-logged condition to extreme water-logging, on the total productivity and protein content of *Aloe vera* (L.) Burm. *f.* was estimated as it was considered to provide valuable information in large scale cultivation of the species for better production and yield of *Aloe vera*.

2. Materials and Methods

Plantlets of different age groups of *Aloe vera* (grown for different periods under natural conditions), were selected for the experiment as sample 'P'- Premature plantlet (20 days old), sample 'Y'-Young plantlet (40 days old) and sample 'M'- Mature plantlet (60 days old). All the three samples were planted in the experimental garden at G.B.P.U.A and T, Pantnagar, Uttarakhand.

All samples were transplanted *ex-situ* under similar conditions and treatments within equidistant spacing of 15 cm^2, to ensure uniform nutrient distribution for experimental accuracy. The tests were conducted in a completely randomized design with four replications. The overall plant yield (productivity) and protein content in various level of water stress conditions were assessed *viz.* T_0 (water-logged condition), T_1 (Daily-water supply), T_2 (Alternate-days water supply), T_3 (bi-weekly water supply), and T_4 (Once-a-week water supply). The plants were harvested after 10 months of normal growth period (including their growth time in natural conditions). For estimation of total productivity the harvested plants were weighed on digital balance (Sartorius CPA225-D, Germany).

Proteins were estimated by using the Bradford dye binding method [17] as follows – For protein extraction, 1 gm leaf of all three samples (P, Y and M) with 2 ml extraction buffer in a chilled pestle-mortar. The homogenate was centrifuged at 10,000 g for 20 min. at 4°C and supernatant collected and stored at –20°C. For protein quantification 100 mg CBB G-250 was dissolved in 50 ml ethanol. To this 100 ml orthophosphoric acid (85%w/v) was added and the volume made up to 1litre with double distilled water. The solution was filtered and stored at 4°C in dark colored bottle.

The Stock solution (1 mg/ml) was prepared by dissolving BSA in Phosphate Buffer Saline (PBS). For this, 12 glass test tube were taken having 5, 10, 20, 40, 60, 100 μl of BSA in duplicate. Double distilled water was added to make volume up to 300 μl. 3 ml of dye was added in each tube and incubated for 5 min at room temperature. The absorbance of all samples was observed at 595 nm. Standard curve was drawn between the BSA concentration and absorbance. The amount of protein was calculated in the unknown sample with the help of standard curve by using the graph. Total protein was estimated by Bradford's method and quantified by plotting a standard curve of BSA as a protein standard for single dimension SDS-PAGE analysis.

The expression of protein profiling was done by Laemmli [18] procedure, with few modifications. The stacking and separating gels, staining and destaining solutions were prepared according to the standardized protocol of Laemmli [18]. For the protein profiling 20 µg of protein was loaded in each well for each treatment and observed banding pattern manually. Leaf protein profiling of different T_4 water treatments is as shown in Plate 1. The relative molecular size of protein bands was examined by comparing the bands with protein markers with range between 14.3–97.4 kDa.

3. Result

This experiments in different combination of water treatment in the three samples of *Aloe vera* (P, Y and M) plants indicate a remarkable variation in their productivity and (gm/plant) and protein content (mg/gm) as follows:

3.1 Total Productivity

(i) In 'P' samples the total yield under different treatments was as follows - T_0-95.6 gm/plant, T_1-101.02 gm/plant, T_2 was 151.02 gm/plant T_3-252.1 gm/plant and T_4-321.09 gm/plant (Fig.1). The maximum yield was obtained from the treatment T_4-321.09 gm/plant and minimum in T_0-95.6 gm/plant.

(ii) In 'Y' samples the total yield under different treatments was as follows - T_0-99.8 gm/plant, T_1-110.00 gm/plant, T_2 was 171.20 gm/plant T_3-286.5 gm/plant and T_4-350.00 gm/plant (Fig. 2). The maximum yield was obtained from the treatment T_4-350.00 gm/plant and minimum in T_0-99.8 gm/plant.

(iii) In 'M' samples the total yield under different treatments was as follows - T_0-101.20 gm/plant, T_1-121.09 gm/plant, T_2 was 256.8 gm/plant T_3-303.2 gm/plant and T_4-371.29 gm/plant (Fig. 3). The maximum yield was obtained from the treatment T_4-371.29 gm/plant and minimum in T_0-101.20 gm/plant.

(iv) The cumulative results of all treatment revealed the best response under the T_4 treatment in all three 'P,Y and M' samples as 321.09 gm/plant, 350.00 gm/plant and 371.29 gm/plant respectively, whereas a diminished response was observed in T_0 Treatments as 95.6 gm/plant, 99.80 gm/plant and 101.2 gm/plant respectively (Fig. 4).

3.2 Protein Estimation

The T_4 samples which responded best to the water supply conditions with maximum productivity were selected to estimate the protein yield. In the three samples - 'P', 'Y' and 'M', the protein of T_4 was as follows - 120.5 mg/gm in 'P', 121.6 mg/gm in 'Y' - and 123.25 mg/gm in 'M'. The maximum protein was obtained from the treatment

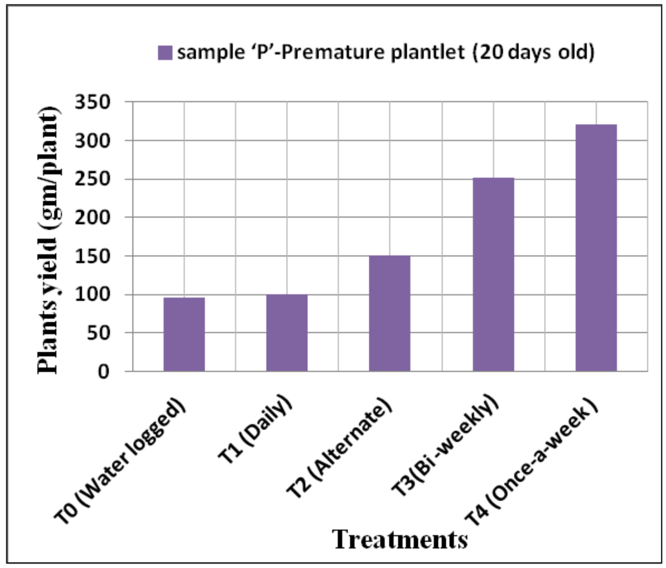

Fig. 1. Productivity variance of *A. vera* by different water treatment in sample 'P'- Premature plantlets (20 days old plant)

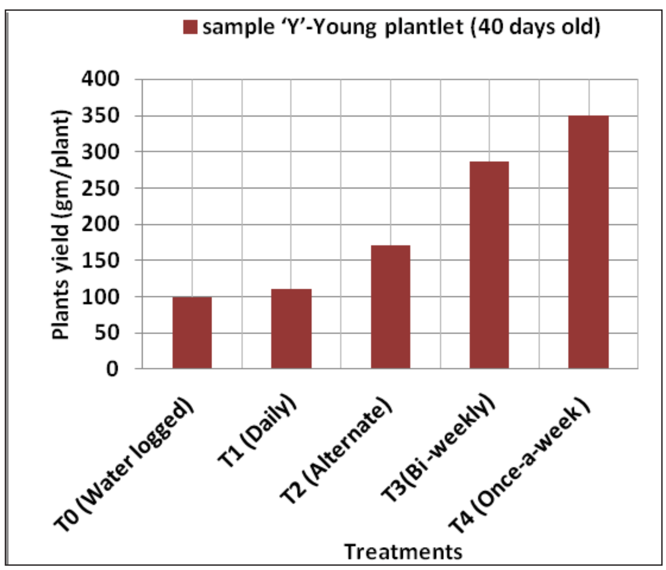

Fig. 2. Productivity variance of *A. vera* by different water treatment in sample 'Y'- Young plantlets (40 days old plant)

sample 'M' -123.25 mg/gm while minimum content was in 'P' -120.5 mg/gm (Fig.5).

The relative molecular size of protein bands revealed that the protein bands were more intense and concentrated under T_4 treatment for sample 'M' and were less intense and feeble under the T_4 treatment for sample 'P'. There were no bands found with protein marker of 29 kDa for all water treatment and also there were no bands produced between 66 to 97 kDa protein marker at sample 'P' and sample 'Y'. The strongest protein bands with the molecular mass 66 kDa were observed in sample-'M'. The light color bands were observed at range 20–29 kDa for all samples 'P', 'Y' and 'M' (Photo plate. 1).

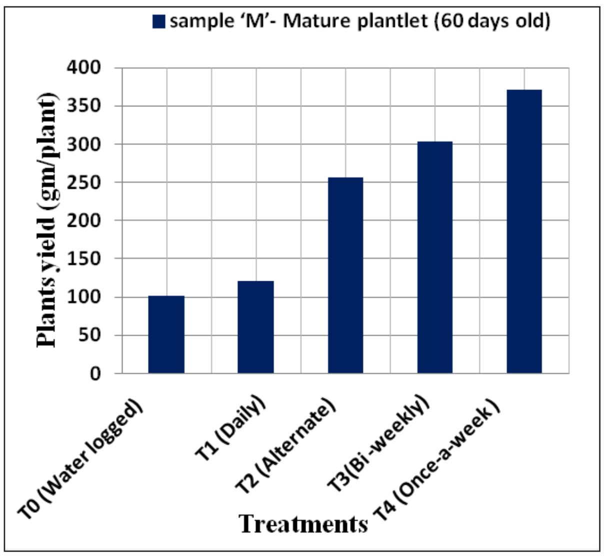

Fig. 3. Productivity variance of *A. vera* by different water treatment sample 'M'- Mature plantlets (60 days old plant).

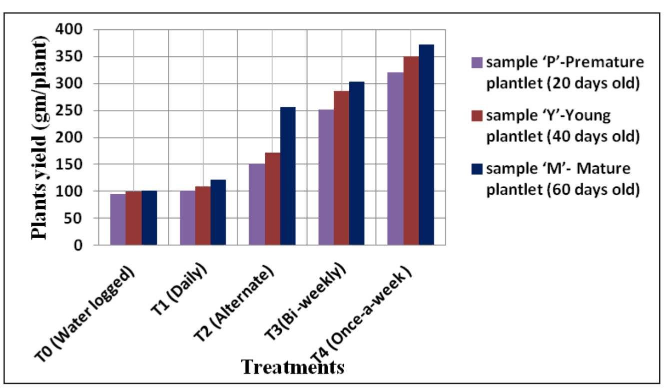

Fig. 4. Productivity variance of *A. vera* by different water treatment in sample 'P', 'Y' and 'M'.

4. Disscussion and Conclusion

The crop production of *Aloe vera* plants depends on many factors such as the climatic, edaphic, irrigation, soil water availability for the root etc. the most important of all these is the water content in soil relative to the atmospheric evaporative demand. The soil water retention capacity and water content determines the metabolic pathway of *Aloe vera*. Since *Aloe vera* is a xerophytic plant their stomata open at night and close

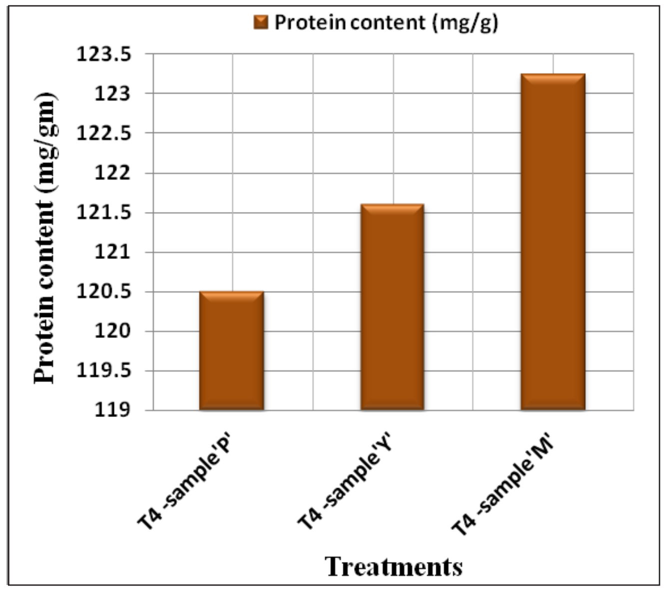

Fig. 5. Protein content variance of *A. vera* in treatment T_4 for sample 'P', 'Y' and 'M'.

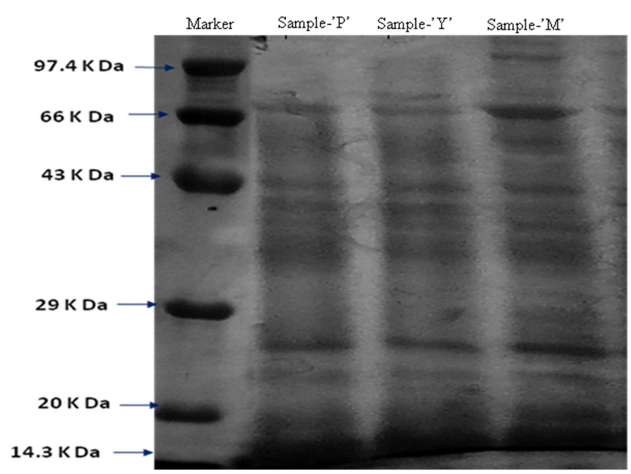

Photo plate 1. Expression of protein profiling of *A. vera* treatment T_4 for sample 'P', 'Y' and 'M' during the day, consequently, all exogenous gas exchange occur at night. Stomatal closure inhibits evaporation thereby maintaining the moisture within tissues and allows the plant to survive in water scarcity. This plant undergo CAM metabolic pathway for conserving water within the parenchymatous tissues to outcompete drought conditions. This fact is expressed through the experiment conducted which shows that water logged soil (T_0) is detrimental to the survival of the xerophytic *Aloe vera* plant. The excess water leads to water-logging of parenchyma cells, an oxygen stress is created around the root surface restricting the respiratory pathway, nutrient uptake mechanism and other critical root functions. This has a negative impact on the biomass production and protein content of the plant and causes damages to the normal photosynthesis pathway (CAM). Hence protein content is also minimal in these plants. Under weekly water supplies (T_4 treatments), the biomass yield (productivity) was found to be maximum with highest protein composition. The total productivity is therefore shown to be directly correlated to the protein content. Water supply in this type of irrigation is provided once and then cut off for the subsequent six days. The weekly water supply is shown to be optimal for best yield of *Aloe vera* in which water absorption is inversely proportional to the soil-water content. Hence in less soil-water, the growth, productivity and efficiency of resource allocation follows the law of normal distribution. However, the reverse happens under excess water in T_0 treatment samples with negligible growth responses and minimum yield in water-logged soil suggesting that water-logged soils are detrimental for growth and sustenance of *Aloe vera*. It is further shown that a continuous or daily supply of water in xerophytic *Aloe vera* (T_1) also hampers the yield, which gets minimized due to the oxygen stress condition created in the roots which restricts water absorption and oxygen deficiency which results in reduced gaseous exchange. The normal oxygen content gets confined to the most superficial portion of the soil, leading to root anoxia as the oxygen available in these regions get quickly consumed by the soil microbes, inhibiting the absorption and transport of water and salts into the root [19, 20].

Under alternate water supply condition (T_2) the excess water supply is minimized to some extent resulting in a better yield, this is followed by increase in the yield and protein content T_3 condition when water depletion period is increase to three days.

The above experiments reveal that the maximum production and protein content of *Aloe vera* plants is obtained under conditions when the soils are watered at a gap 6 days (once in a week; T4 treatment). In this process plant is flushed with water on 1st day and left unwatered for subsequent days. During this time of six days, when water is depleted, the plants utilized CAM pathway for slow carbon assimilation and increase water use efficiency for photosynthesis, which permits the plants to complete their life cycle as the roots are not exposed to oxygen deficiency for normal respiration. However, since water is essential for root growth that depends on cell elongation, to create a force that causes expansion [22], the plants need to be watered at regular intervals. Any further water depriving is therefore undesirable for normal root growth.

Our results also suggest that the Water Uptake Efficiency (WUE) of *Aloe vera* is best under the low water levels as indicated by the highest productivity (in treatment T_4) as compared to other treatment $T_{0,}$ T_1, T_2, T_3. These differences in the productivity and protein contents were based on the water quantity consumed by the root, WUE of plant and the amount of water utilized by the plants from the available water. The excess water provided within short intervals as in daily-watering (T_1), alternate-day watering (T_2) and bi-weekly (T_3), water is more than what is required for xerophytic plant and hampers the root for water absorption and subsequently affected biomass production while the weekly water supply yielded a good productivity and protein content. The results indicated that at individual plant level, the values of WUE of *Aloe vera* is comparable to the xerophytic (*Opuntia* and *Agave*) species [21]. These values also confirm the high WUE of CAM plants when compared to C3 and C4 species and the rate of carbon assimilation by CAM plants to be 1/2 of C3 and 1/3 of C4 plants. Confirming the observations of Martinez et al. [22] it is confirmed here that high yields under conditions of limited of water availability are associated with low WUE values due to high rates of evapo-transpiration. Further, the characteristics associated with a low potential yield, such as small plants or a short growing period, are associated with high WUE since they reduce the use of water [23–25]. Our results show that an increased yield and protein constitution of *Aloe vera* is obtained under intermittent, low water supplies, for once a week as water uptake is directly associated with low water use efficiency.

From the studies it is evident that the plant yield or the total productivity of the highly economic, medicinal plant species *Aloe vera* is dependent upon the irrigation pattern of the crop. Excess water is detrimental for sustenance and productivity of the crop. As the water supply is detained the crop yield increases and the best results of maximum productivity and high protein content are obtained when the juvenile plantlets are initially left under natural conditions with average watering for 60 days, followed by weekly irrigation for the ensuing eight months of harvesting period. This irrigation method is therefore recommended for boosting the production of *Aloe vera* crops.

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