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Impact of Drought Stress on Active Secondary Metabolite Production in *Cichorium intybus* Roots

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ABSTRACT

Chicory (*Cichorium intybus*), a species of the Asteraceae family, is a biannual crop. Chicory is grown for a number of reasons in different countries. Dried chicory root extract contains inulin as an active compound, which is widely used as a prebiotic agent with antioxidant properties. Chicory was cultivated under low tunnels in blocks and after three month of normal irrigation drought conditions were applied by irrigating after every 14-, 21-, 28- and 35 days of irrigation intervals. Chicory roots were harvested at the age of six month and inulin was extracted by batch extraction method. Inulin concentration was measured with calorimetric method. The results showed that root growth and accumulative inulin yield decreased as drought period increase. But the total inulin percentage in chicory roots increased by mild drought stress and decrease with severe drought stress. Our research highlights the impact of drought stress on chicory roots growth and inulin production in it.
KEY WORDS. Drought, Chicory root, Inulin.

INTRODUCTION

Drought is the most acute stressor of plant growth and recently it is becoming more serious issue of the world and desertification is increasing day by day [1]. In fact, rapidly increasing of desertification on a global scale is currently affecting more than 10 percent of arable land which causes more than 50 percent of yield reduction in major crops [2,3].

Chicory belongs to family Asteraceae and its botanical name is *Cichorium intybus*. Chicory is grown at large scale in different temperate regions all over the world, it is basically native to western Asia, Europe and central Russia [4]. In different parts of world it is cultivated for different purposes. The extract of dry roots of Chicory contains 98 percent inulin approximately and 2 percent other chemical compounds. In fresh roots of Chicory inulin is present between 13 to 23 percent by weight [5]. Inulin is a naturally stored polysaccharide in chicory roots[6]. Due to its antioxidant properties, inulin is used as agent for prebiotic functions [7]. In colon, the good bacteria like lactobacillus and Bifidobacterium are selectively stimulated by Inulin that is help full in prevention of disease and take a main part for maintenance of good health [8]. Inulin is used at large scale as alternate of sugar and lipid compounds in mostly food products. It is also use in bread and other products of food to increase the contents of dietary fibers [9]. Inulin is a good substitute of fat and sugar and also has a main advantage of having very less caloric rate and also has many functional characteristics. In organisms it is act as similar to dietary fibers and takes part in upgrading the conditions of gastrointestinal conditions. Inulin also optimize the beverage's texture and also enhance the viscosity of beverages with low caloric value as well as allow low quantity of fat to spread more on surface also give spreading ability to product without fat like in chocolates, mousses, yogurts, salad dressings, etc. [10].

In several parts of the world, root chicory is now cultivated under irrigation, but a better knowledge of its behavior under water stress conditions should allow the minimal water requirements of this species to be defined and thus allow its culture to be extended to regions where irrigation is not technically feasible or economically justified. Although responses to low temperature have been extensively studied in this species, mainly with respect to vernalization [11,12,13], the impact of water

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stress has received less attention. De Roover et al. (2000) studied the impact of water stress at the seedling stage and demonstrated that water shortage increased glucose, fructose, and sucrose concentrations in the roots and leaves of stressed plants, leading to increased fructan concentrations in the roots[14]. Using a field approach, Monti et al. (2005) demonstrated that water shortage had only a limited impact on yield, leaf photosynthetic capacity was poorly affected by water availability, and fructan chain length was not affected by the water regime [15]. Previously, the severity and timing of drought were reported to strongly influence the sequence of plant reactions to water shortage [16, 17], but the drought impact on inulin production is missing. Keeping in view all above facts the present study was conducted to evaluate influences of different drought on inulin production in root chicory.

MATERIALS AND METHODS

Experimental design

Chicory was sown under low tunnel in the square number 32, University of Agriculture Faisalabad. Land was divided in to 3 blocks, each block with 5 rows of chicory plants. Distance between each row was maintained about 1 meter. During first three months after cultivation of chicory, irrigation was applied normally after each 7 days interval and drought conditions were applied by irrigating after every 14-, 21-, 28- and 35 days of irrigation intervals. Crop was harvest as a whole with roots after blooming at the end of 6^{th} month. Fresh roots weight and length were measured, roots were shade dried and roots dry weight was measured. Dry roots were crushed and grounded by an electric grinder.

Inulin determination

Chicory root powder was use as the starting material. The prepared sample, with an approximately 5 % moisture content, was stored in a dry container for further use. Batch extraction was perform at 70°C with continuous stirring. Distilled water was used as solvent for inulin extraction. The liquid inulin solutions obtained at the end of each experiment was filter through a cloth to remove large root pieces and then through a Whitman filter paper to remove smaller particles. For primary extract purification and concentration, suspended fine particles were remove by filtration through silica gel-chitosan bed. The effluent was concentrated by evaporation. Inulin contents of the raw extracts was measured as follows: inulin extract (0.1 g) was hydrolyzed by 0.8 MHCl (1 ml) at 900 C for 1 hour, after which the hydro lysate (150 µl) was tested for reducing sugars using the fructose standard curve [18].

Data analysis

Correlation between desired factors was carried out using corragram package. Impact of drought on plant root growth and inulin production was tested using linear regression analysis and all figures were plotted using ggplot2 package. All the data was analyzed by R 3.2.3.

RESULTS

Impact of drought on chicory Roots growth

Chicory roots grew more during non-drought condition and root's fresh weight increased. Plant roots fresh weight decreased as the drought period increased. Maximum weight of chicory plant roots were observed when irrigation interval is after just 7 days and minimum fresh roots weight were noted during 35 days of irrigation interval. Chicory plant root length of decreased as the interval between irrigation time increased (Table 1). Plant root fresh weight and root length is negatively correlated to irrigation duration (Figure 1).And regression analysis reveals that the water stress has a strong impact on chicory root growth both is weight and length (Table 2).

Impact of drought on Inulin content and accumulative yield

Inulin Percentage in roots of chicory plant increase as the interval between irrigation time increases from 7 days to 21 days. After that inulin content in chicory plants began to decrease. Maximum inulin percentage in roots of chicory plant was noticed when there is irrigation after just 21 days and inulin percentage in roots of chicory plant begin to increase as drought period increase. Inulin Percentage in dry roots of chicory plant was minimum when plants were frequently irrigated after 7 days interval. Accumulative Inulin yield in roots of chicory plant increased from 7 days irrigation interval to 21 days irrigation interval started to decrease in 28 and 35 days irrigation interval (Table 1).

Table 1: Average ± standard deviation of final measurements

Irrigation interval(days)	Inulin%(Dry root)	Inulin%(Fresh root)	yield(g)	Fresh Weight(g)	root length(cm)
7	67.3±1.9	9±0.32	6.7±0.12	75.2±0.32	25.6±0.6
14	77.9±1.1	21±0.4	11.4±0.24	54±0.2	20±0.3
21	90.93±0.33	23±0.2	15.2±0.3	48.7±0.2	16.3±0.1
28	84±0.2	27±0.8	7.5±0.48	29±0.6	11.6±3
35	81±0.9	21.6±0.2	3.4±0.4	11.5±0.48	5.9±0.16

Correlation analysis reveals that inulin production is positive correlated to drought period but accumulative inulin yield is decreased with increasing drought (Figure 1). Regression analysis reveals that irrigation duration significantly effect on inulin production (Table 2).

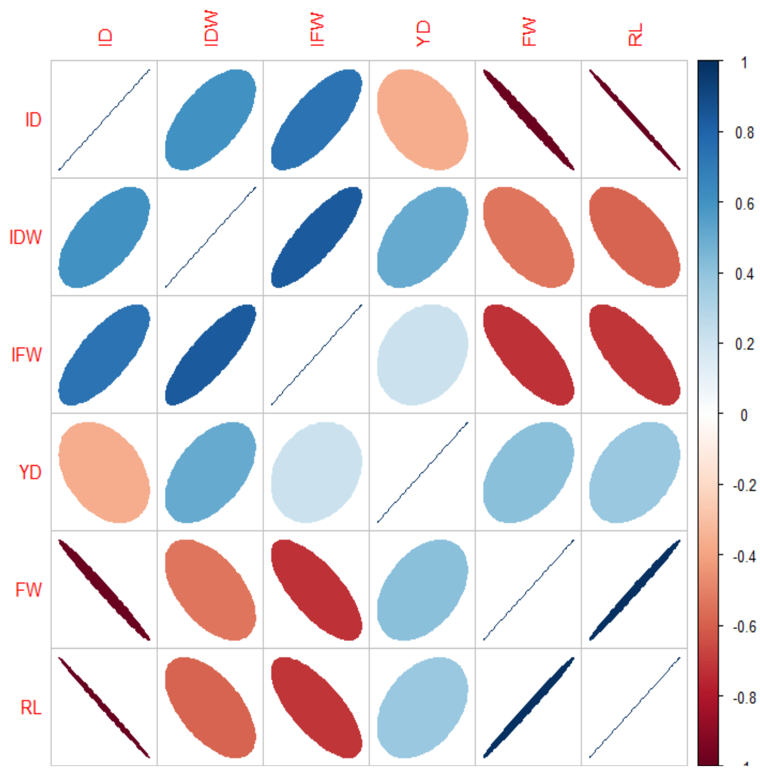

Figure 1: Correlation analysis between irrigation intervals (ID), chicory root fresh weight (FW), root length (RL), fresh root inulin content (IFW), dry weight inulin content(IDW) and inulin accumulative yield(YD).

Table 2: Impact of drought on chicory root growth and inulin content

Attributes	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
IDW	1	538.2368	538.2368	841.6132	>0.001	***
IFW	1	247.6169	247.6169	387.1858	>0.001	***
YD	1	638.5596	638.5596	998.4829	>0.001	***
FW	1	38.14087	38.14087	59.63892	>0.001	***
RL	1	1.690068	1.690068	2.642673	0.138473	
Residuals	9	5.755769	0.63953			

In Table 2 *** represents highly significant values, dry weight inulin content(IDW), fresh root inulin content (IFW), inulin accumulative yield(YD), root fresh weight(FW) and root length (RL).

DISCUSSION

Drought resistance is a major concern in plant research. In several studies conducted under laboratory environments, drought resistance improve the survival rate under fatal conditions that conditions are impossible to maintain under open field. Drought stress checks the plant growth that may help in survival of the plant but it could be considered counter-productive in terms of yield in agriculture [16,17,19]. Carbohydrates produced from photosynthesis are major energy sources and building blocks for production of biomass and its maintenance. Stressed plants accumulates carbohydrates with the reduction in osmotic potential. Water stress accelerated the leaf senescence, despite the leaf senescence process, change in the leaf shape and structure tends to an efficient absorption of photosynthesis light and that efficient photosynthesis allows the plant to continue sugar biosynthesis to fulfil the requirements of reserves accumulation in the plant roots. This work presents that chicory was drought conditions strongly effects chicory growth and inulin contents (a carbohydrate). Growth inhibition was noticed in roots length, size and weight [20].

Moreover, the inulin concentration was also increased by mild drought indicates that sugar transport from leaves (source) to roots (sink) was not severely hindered under mild drought. In severe drought condition, leaves abscission hinders photosynthesis and halts the reserves transport. According to Schittenhelm (1999), water deficit is a major factor decreasing chicory root yield [21]. Chicory root growth drastically decreased by both mild and severe drought, but not biosynthesis of inulin [22].Our study represents that the inulin concentration in chicory roots may increase in mild water stressed plants and decrease with severe drought condition.

Conclusions

Chicory shows high level of drought resistance, which could be reason of its adoption in Mediterranean origin. Plant growth retardation could be one of its resistance strategies but a major causes of total inulin yield reduction. Further research is therefore needed to identify the main inhibitory factors of root growth in chicory.

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