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How does water supply affect *Taraxacum koksaghyz* Rod. rubber, inulin and biomass production?

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ABSTRACT

Taraxacum koksaghyz Rodin (TKS) has been many times proposed as an alternative source for rubber and inulin production, but it is not being used at commercial scale due to the excessive production and extraction costs. We have studied the needs of water supply to find the best conditions for optimum production in order to facilitate the establishment of TKS as a potential, natural and renewable source for rubber and inulin production. Three irrigation regimes, including two different dosages and a non irrigated control, were evaluated in a field trial in Northern Spain. Only a significant influence of the irrigation regime on percentage of TKS rubber contents was observed, while no significant effects on inulin contents, biomass production and other morphological traits were detected. Additional water supply for optimal growth seems not to be necessary for TKS optimum development under local weather conditions, but further studies at dryer locations should be performed. A strong positive correlation was observed between root and leaf dry and fresh weights and a weak to moderate negative correlation between percentages of rubber and inulin contents.

1. Introduction

Taraxacum koksaghyz Rodin (TKS) has been suggested in many occasions as a possible alternative crop for good quality, natural rubber and inulin production (Krotkov, 1945; Whaley and Bowen, 1947; Whaley, 1948; van Beilen and Poirier, 2007a,b). It was already used for rubber production for army vehicle tires during the 2nd World War by the Russian Government (Mooibroek and Cornish, 2000), but its usage and future research was abandoned due to the reopening of the commercialization of *Hevea brasiliensis* natural rubber at the end of the war (van Beilen and Poirier, 2007b). TKS inulin has been never extracted in an industrial scale, even when its properties and quality are well known. It still needs more research on its economic viability.

A patent was registered by Wade and Swiger (2011) stating that all parts of the plants are profitable. Inulin and rubber can be extracted at the same time, making this crop economically viable.

Natural rubber is a biopolymer consisting of isoprene units $(C_5H_8)_n$ linked together in a 1, 4 *cis*-configuration. The molecular weight of an isoprene monomer in natural rubber (C_5H_8) is 68 Da. (van Beilen and Poirier, 2007b). Natural rubber is considered to be one of the most versatile agricultural products. It is a strategic material, being utilized in the manufacturing of more than 40,000 consumer products including more than 400 medical devices, surgical gloves, and aircraft tires (van Beilen and Poirier, 2007b).

Rubber is present in leaves and roots of TKS. However, leaf rubber has a low quality due to the associated resins. In contrary, the root rubber represents a high quality product. This is the reason why only root rubber is being pursued for commercial aims (Whaley, 1948). Until now no irrigation experiments for the evaluation of TKS rubber production have been published.

Another promising, alternative natural rubber source which is already being commercialized is guayule (*Parthenium argentatum* Gray). This species is known to be a semiarid and drought-tolerant shrub, but must be irrigated for maximum sustained production. From 1000–1300 mm of water from rainfall and/or irrigation per year is needed to attain maximum production. Most of the literature indicates that decreasing irrigation results in increasing rubber content, but decreases also shrub biomass (Foster and Coffelt, 2005).

Inulin is a natural renewable polysaccharide with a significant number of diverse pharmaceutical and food applications (Barclay

Abbreviations: TKS, *Taraxacum koksaghyz* Rod; R_0, R_1, R_2, irrigation regimes; RDW, Root dry weight; RFW, Root fresh weight; LDW, Leaf dry weight; LFW, Leaf fresh weight; LL, Leaf length; LfN, Leaf number; N, Number.

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et al., 2010). It is a polydisperse fructan with a degree of polymerization (DP) between 2 and 60 or higher. The fructosyl units are connected by (2→1) linkages with an end glucose residue. The inulin DP depends upon many factors such as plant source, climate and growing conditions, harvesting time and storage conditions (De Leenheer and Hoebregs, 1994; Coussement, 1999). Even the type of the tissue from which it is extracted has an influence (Van den Ende et al., 2000). The DP determines the type of use of the inulin, either for food or for pharmaceutical purposes. Inulin with low DP can be added to food as a low calorie sweetener, whereas inulin with higher DP can be used as a fiber-type prebiotic with several health promoting effects (Flamm et al., 2001). In plants, inulin-type fructans mainly occur in dicot species belonging to *Asteraceae* (Van Laere and Van den Ende, 2002). Important species are chicory (*Chicorium intybus* L.), artichoke (*Cynara scolymus*), Jerusalem artichoke (*Helianthus tuberosus* L.), dandelion (*Taraxacum officinale*), dahlia (*Dahlia variabilis*), yacon (*Polymnia sonchifolia*) (Van Laere and Van den Ende, 2002; Wilson et al., 2001; Schütz et al., 2006) and Russian dandelion (TKS) (Gorham, 1946; Krotkov, 1948; Mikhlin and Akhumbaeva, 1955). Even though TKS is still in a pre-commercial stage, the use of its inulin has been studied for the production of pharmaceutical preparations and dietary supplements (Schütz et al., 2006), as well as for producing biofuels such as ethanol and butanol. (Buranov, 2010; OARDC, 2014; OMAFRA, 2014; AGMRC, 2014). Also the residual root bagasse and leaves have been proposed for cellulosic biofuel production, helping in this way to promote the commercialization of TKS as a viable natural rubber source (Gorham, 1946; Wade and Swiger, 2011; Bharathidasan, 2013).

In 1948 Krotkov already mentioned that during the first and second year of growth there is a steady increase in the inulin content of TKS roots. It goes from the beginning of the vegetative growth in spring until the middle of the flowering period. Hendry assigned in 1993 temperate climate zones with seasonal drought to fructan-accumulating plants. However, at this moment the relation between fructans and mechanisms for drought and freezing protection was not clear. Later on, in 2001, Wilson et al. directly related fructan metabolism with the exposure of plants to cold temperatures and moisture stress. They also evaluated seasonal changes of fructans in dandelion roots and correlated these changes with seasonal fluctuations in soil temperature and precipitation. They concluded that 50% of fructan variation can be explained by soil temperature and 15% of the variability was attributed to differences in rainfall.

Numerous are the studies on chicory, Jerusalem artichoke and other inulin producers. The objectives were to diminish production costs and rising plant efficiency through the reduction of water supply (De Maestro et al., 2004; Monti et al., 2005; Vandoorne et al., 2012). However, this kind of research is almost inexistent in TKS. In 1947, Whaley and Bowen wrote a compilation of studies on TKS where also some irrigation experiments were mentioned, but no relation with rubber and inulin production was evaluated.

In the present study three irrigation regimes were assayed in a field in Northern Spain during 2010. The effects of water supply on biomass production, inulin and rubber contents and on some other morphological characters were analyzed.

2. Materials and methods

2.1. Plant material

A controlled cross between TKS parent 237-2 (low rubber producer) and parent 194-1 (high rubber producer) was performed by KeyGene (Wageningen, The Netherlands) in 2009 in order to establish a mapping population. This progeny was multiplied *in vitro* by KeyGene and five plantlets of 68 genotypes were sent to NEIKER Tecnalia. Plantlets were raised in pots with turf in the greenhouse and transplanted after one month to the field in 2010. After flowering, inflorescences were collected from all genotypes, dried at room temperature and seeds were extracted. Seeds were kept in the refrigerator at 4 °C for some months. A random mixture of these collected seeds was used for the irrigation experiment. They were spread in wet paper beds with nutritive medium at 18 °C for one week, until germination occurred. In mid-April 2011 the seedlings were planted in a shaded greenhouse chamber and young TKS plants were raised for planting in the field trial. Seedlings were irrigated manually. No special temperature and light conditions were applied (light range of 270–340 (μmol/m^{2}s); Tª range 15–25 °C).

2.2. Field trial

The irrigation trial was performed in the experimental field of the "Model Farm of Arkaute" (42° 51′ 0.29″ N −2° 37′ 21.59″ W; 517 m elevation) belonging to NEIKER Tecnalia, close to Vitoria-Gasteiz (Northern Spain), during the year 2011. The annual accumulated precipitation was 547 (L/m^2), mean Tª was 11.6 °C and average humidity 86.8%. The texture of the field was classified as Loam.

The trial was set up as a randomized block design with four repetitions. The analyzed main factor was irrigation regime with three levels (see below). Elementary plots had a width of 1.5 m and were 3 m in length. The soil didn't get any extra fertilization. Ridge planting was practiced and a green anti weed MIPED mesh was used. The seedlings raised in the greenhouse were planted beginning of June at a depth of 7–8 cm. They were planted in four rows, nine plants per row, corresponding to a plant density of 40×33 cm^2. After planting, 30 min irrigation was applied. In order to avoid moles, an ultrasound mole chaser was used.

Harvesting was done end of October. Four roots from the center of each elementary plot were dragged out manually, washed and full plants were kept in a cool chamber at 4 °C until processing up to four days later.

2.3. Irrigation regimes

The dosage calculations for irrigations were based on the performance of an hydrological balance in which hydrological contributions (effective precipitation, supplied irrigation and irrigation system efficiency) and the hydrological usage (evapotranspiration) by the crop were taken into account. The method used for calculations of evapotranspiration was based on the estimation of the crop evapotranspiration (ET_c) depending on the reference evapotranspiration (ET_0), estimated by the FAO 56 Penman-Monteith equation and the adoption of a dual crop coefficient (FAO, 1998). The basal crop coefficient (K_{cb}) of *Beta vulgaris* was taken as reference, due to its close similarity to TKS. The model uses a stress coefficient (K_s) that diminishes the K_{cb} value when the hydrological deficit of available water ranges the value from which the plant starts to suffer hydrological stress. K_e (Evaporative coefficient) represents the evaporative rate from the first centimeters of soil (10–15 cm), in variable humidity conditions from this level. Based on these considerations and the local conditions a reference dosage was calculated for TKS. Three irrigation regimes were applied in the field trial: R0 = no irrigation; R1 = half of the reference dose; R2 = full reference dose. The supplied water dosage was adjusted weekly.

The irrigation system was formed by an irrigation hose with exudation (CT-12 Agro, from Poritex) connected to a pressure regulator (0.7 bar) and a timer. 15 l/m^2 $\times$ h were dispensed by the system.