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Short communication

Adaptability of two accessions of *Geranium macrorrhizum* L. to drought stress conditions

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ABSTRACT

Geranium macrorrhizum L. is a herbaceous perennial with underground stems (rhizomes) naturally found in the Balkan countries where it is known for its phytochemical properties. Despite its potential, there are no references to cultivation of this species to produce essential oil; to date only wild plants have been harvested. The purpose of this study is to compare cuttings of *G. macrorrhizum* taken from different starting material in terms of resistance drought stress and their morphological and physiological growth responses. Cuttings from two different origins (n = 5), the English variety called 'Bevans' (BV), and a wild geranium population from Hungary (GH) were studied. Water potential (ψ) was measured with a Scholander chamber for one month (August) during which time the plants did not receive any water. Once the water potential curve was obtained, the morphological and functional components of the growing process were measured in 5 plants from each accession: leaf area ratio (LAR), leaf mass fraction (LMF), root mass fraction (RMF), water stored in the aerial part (WSL) and water stored in the roots (WSR). Both accessions maintained water potential unaltered for 20 days, GH losing more water during the experiment. Results show that the LAR for GH plants was 3 times higher than for BV plants (631.7 and 247.3 respectively). Regarding biomass distribution, GH plants had a greater LMF (0.41) and consequently a higher WSL in leaves (37.96). BV plants exhibited a higher RMF (0.87). Despite being the same species, the two accessions show important morphological and physiological differences which are most likely the result of the selection process to achieve the 'Bevan' variety used for ornamental purposes.

1. Introduction

Geranium macrorrhizum L. is a herbaceous perennial with underground stems (rhizomes) naturally found in the Balkan countries where it is known for its phytochemical properties, mainly containing the sesquiterpene germacrone (Chalchat et al., 2002; Lis-Balchin, 2002). This species is also used for ornamental purposes because of its pink or white flowers (Chalchat et al., 2002; Ivancheva et al., 1992; Leslie, 1993; Lis-Balchin, 2002). In countries such as the United Kingdom and The United States, ornamental varieties have been developed to especially withstand bitter cold weather and are able to tolerate low light and water.

Over the last few years the species has been studied due to its phytochemical properties (Lis-Balchin, 2002). Despite its potential however, there are difficulties in producing essential oil for commercial purposes (Weiss, 1997) especially due to the limited availability of plant material from wild-growing populations which are only found in a relatively small geographical area. As a result, the oil from wild species is expensive and quality control is lacking.

Phenotypic plasticity is the ability to produce different phenotypes in response to environment differences (Pigliucci, 2001). One example is the response to the shade influence when plant plasticity can be expressed in fewer but larger and thinner leaves (morphological plasticity); or changes in biomass distribution between aerial parts and the root system (physiological plasticity) (Valladares, 1999). This plasticity is accepted as a characteristic that is itself under selection and of ecological and evolutionary significance (Forde, 2009; West-Eberhard, 2005). Such plasticity is now recognized as being heritable (Tucic et al., 2005; Weijschedé et al., 2006) and can be altered by artificial selection experiments (Garland and Kelly, 2006; Teuschl et al., 2007).

Variation in some traits occurs passively either when growth is stunted by limited resources or as a result of genetic correlations with traits that are under selection (Van Kleunen and Fischer, 2005). Such trait variation can be described as passive plasticity and is not likely to

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correlate directly with robustness (Caruso et al., 2006). In other cases a plastic response can actually reduce robustness. For example, Scots pine (*Pinus sylvestris* L.) trees tend to adopt higher transpiration rates in warm dry climates resulting in lower water use efficiency which is counterproductive (Poyatos et al., 2007).

The aim of this study is to analyze how *G. macrorrhizum* adapts to Zaragoza, a city that is representative of the region of Aragon with average annual precipitation of 550 mm. The performance of plants from different starting material was evaluated in terms of dealing with drought stress and morphological and physiological growth response.

2. Material and methods

Cuttings from two different origins were used for plant analysis:

- The ‘Bevan’ Variety (BV)selected for ornamental purposes in the UK (CPVO, 2014)
- Native material from Hungary (GH) collected at Budapest University (Budapesti Corvinus Egyetem).

All propagation assays were performed at the Unidad de Recursos Forestales (Centro de Investigación y Tecnología Agroalimentaria de Aragón – CITA located in Zaragoza, Spain) (41° 39′ 23″ N and 0° 52′ 36″ W; altitude 218 m).

Cuttings obtained from rhizome division, were grown in a greenhouse for the first five months and then moved to a lath house for the rest of the year to simulate their natural environment. They were kept in individual pots (5 l volume) containing a commercial mixture (HuminSubstrat® N3 containing 90% turf, 10% clay; pH 6; NPK 14:16:18, conductivity 35 mS m^{-1}). Watering and weed control was performed as needed (manually with no chemical treatment). A total 40 cuttings (pots) were used for each of the two accessions (one cutting per pot). Statistical analysis was t-student test using Statgraphics (version 15.1.0.2) to compare the means.

2.1. Response to drought

One week before the trial began, 5 random pots from each accession (BV and GH), were placed in a dry, roofed lath house protected with an anti-aphid mesh (40% light reduction) and preventing any external water supply.

In order to estimate water, plant water status was measured based on water potential (Jones, 2007; Slavík, 1974). Water potential (ψ) describes the energy status of water in plants and is expressed as potential energy per unit volume, the units being pressure expressed as MPa or bars. The most widely used method to measure such pressure is the Scholander pressure-chamber (Scholander et al., 1965; Turner, 1988).

The first measurements were taken in August (summer) in plants that were watered that same day when the water supply was cut off for all plants. Measurements were taken weekly for 6 weeks, 4 leaves from each of the 5 plants being sampled per accession (20 replicates each), always at 9:00 a.m. (Fig. 1) using a Scholander pressure-chamber.

2.2. Morphological and functional components of the growth process

Five pots with plants from each accession (BV and GH) with similar morphology were used.

The morphological component was measured by the leaf area ratio (LAR; $m^2\ kg^{-1}$), the ratio between the leaf area and total weight of the plant. The software Image J, version 1.49 (Rasband, 2007) was used for this purpose.

The functional component was measured asthe biomass distribution to the different plant parts and leaf area formation (Poorter, 1989). Measurements used were leaf mass fraction (LMF – leaf biomass/total biomass; kg kg^{-1}); and root mass fraction (RMF – root biomass/total biomass; kg kg^{-1}).

To calculate the amount of water that the aerial parts (WSL) and roots were able to store (WSR), the leaves and roots ofwell watered plants were weighed before and after drying (70 °C in a drying oven, for 72 h).

3. Results and discussion

The *t*-test to compare the water potential (ψ) means for the two accessions (BV and GH) revealed a significant difference where BV plants loose less water (Fig. 2). Since values are negative they must be interpreted inversely, i.e. higher water potential means less water loss.

Although BV performed better, both accessions were reasonably resistant to drought during the trial, water potential (ψ) not increasing drastically until week 4. Resistance to drought may be partly related to *G. macrorrhizum*’s ability to regulate water loss by closing stomata (Hillováet al., 2016; Zollinger et al., 2006). Heightened stomatal sensitivity is a functional mechanism that allows plants to maintain an ample amount of water during dry periods (Pessarakli, 2002). Stomata gradually close as water stress increases.

The plants lost water slowly, water potential remaining nearly unaltered for 20 days. After day 20 water loss accelerated as indicated by the curve. This is a positive result considering that this plant was transferred from a wet environment to an area with scant rainfall such as Zaragoza (average annual precipitation and temperature of 550–750 mm and 15.6 °C) (IAEST, 2017).

Table 1 results show that the LAR for GH plants is 3 times higher than for BV plants (631.7 and 247.3 respectively). In general, species that allocate more biomass to leaves are able to grow faster but show a negative correlation to root biomass (Antúnez et al., 2001; Wright and Westoby, 2000) which is evident in the following results.

Regarding biomass distribution, the LMF of GH plants is higher (0.41) resulting in higher WSL in leaves (37.96). BV plants exhibited higher RMF (0.87) but not a higher WSR in roots as would have been expected. WSR was similar for the two accessions which could mean that GH plants loose water faster though transpiration as claimed by Grace (1997). In other words, a reduction in the LAR index when water is less available should reduce water loss by transpiration as shown in Fig. 2.

Valladares (2008) confirmed that a higher LAR index promotes growth when water is readily available but can increase transpiration and disrupt the water balance in plants during the dry season thus reducing the probability of survival. Nevertheless, this study shows that *G. macrorrhizum* plants tolerated at least one month of drought in August with high temperatures (13 °C–37 °C), corroborating Hillováet al. (2016) who identified commercial variety of *G. macrorrhizum* that was well adapted to dry conditions making it suitable for conditions of scant rainfall.

These results support our hypothesis that two accessions of the same species can exhibit significant morphological and physiological differences. These differences are most likely the result of the selection process to achieve the ‘Bevan’ variety for ornamental purposes.

Root system differences were already noticeable when rhizomes were divided to obtain cuttings. BV plants featured a typical horizontal rhizome with many shoots and were easily divisible. In contrast, GH plants had a modified rhizome, somewhat of a pivoting system with a few side shoots which were more difficult to divide for cuttings (Fig. 3).

Leaf appearance is also different. GH leaves were larger and thicker, of a dark green color and had a velvet-like appearance. They also recover faster from frost, flowering at the end of April. In contrast, BV leaves are light green and very thin, flowering at mid-May (Fig. 4).

It goes without saying that plant growth is influenced by environmental conditions such as water availability and nutrients. However, the growth index has a genetic component as well. The growth index affected by genetic and the environmental factor has ecological consequences for the natural regeneration of plant populations and the