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Review

Critically endangered endemic *Centaurea tchihatcheffii* Fisch. and Mey. and its propagation possibilities

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Centaurea tchihatcheffii **Fisch. and Mey., is a critically endangered endemic species, which grows on limited area around Golbasi district of Ankara province. It has attractive pink, red, purple flowers, the colours of which change with maturation. It has a high potential as out door ornamental plant and as cut flower. Its natural habitat is continuously on decrease because of uncontrolled plucking, intense construction activities and urbanization. The most important reason why the generation of the species is endangered is strong anthropogenic pressure. Therefore, the protection of this plant is of great importance. Study of propagation possibilities for the protection of this species, is of primary importance. This paper reviews *C. tchihatcheffii,* propagation studies.**

Key words: Critically endangered endemic (CR), propagation, *Centaurea tchihatcheffii* Fisch. and Mey.

INTRODUCTION

The floristic richness and diversity of a country gains importance with its number of rare and endemic 'critically endangered (CR)' plant species (Ekim et al., 2000; IUCN, 2001; Vural, 2006). The endemism rate of Anatolia, which is one of the most important gene centres of the world in terms of endemic plants, has reached 34.4% (Gurbuz et al., 2009). The proportion of endemism and the number of endemic plants are very high when compared with those of other European countries (Erik and Tarıkahya, 2004; Avci, 2005; Ekim, 2006; Parmaksız and Khawar, 2006; Gurbuz et al., 2009). In terms of endemic plant species, Genus *Centaurea* (starthistles, knapweeds, centaureas, cornflower or Gökbas) has 110 endemic species found in Turkey (Anonymous, 2010a). *Centaurea tchihatcheffi* Fisch. and Mey. belonging to this group is critically endangered plant species that grows only on limited scale in the vicinity of Ankara-Golbasi-Mogan-Eymir Lakes, at a distance of 20 km from Ankara. *C. tchihatcheffi* is a very magnificent plant with its colourful flowers and beautiful appearance. The most important reason why the generation of the species is endangered is strong anthropogenic pressure. It is very essential to evaluate this plant species precisely to avoid its extinction. Turkish laws ban trade in critically endangered species, however, due to loopholes in the law, the flowers of *C. tchihatcheffi* are collected for illegal use in cut flower industry in the cut flower markets of Ankara. Limited literature is available on taxonomy, biology, botanical characteristics and its conservation (Bosgelmez, 2005, 2006; Cakarogulları, 2005). Previous studies report propagation of this plant species through plant tissue culture and cuttings (Özel, 2002; Özel et al., 2006a,b). Propagation of the species has remained low in its natural habitat due to high seed dormancy due to embryo and needs a long vernalization to break it (Cakırlar et al., 2005, 2006; Günöz, 2008; Okay and Günöz, 2009). Tipirdamaz et al. (2006), emphasize that better propagation rates could be obtained through development of *in vitro* and *ex vitro* propagation techniques including propagation through seeds. Propagation of this plant through breaking seed dormancy could help in restoration of this ill fated species.

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The scientific classification, history, dissemination areas in the world, and Turkey's flora of *C. tchihatcheffii*

C. tchihatcheffii Fisch. and Mey. is included in the *Magnoliophyta* division, *Magnoliopsida* class, *Asteridae* subclass, *Asterales* order, *Asteraceae* family and

Centaurea genus. There are approximately 182 species in the *Centaurea* genus (Anonymous, 2010b). The *C. tchihatcheffii* is distinguished from the other Centaureas by its specific features.

The specific name of the plant comes from the Centaur, which is a mythologica monster in the shape of a horse with a human head, the existence of which was proposed by Hippocrates. Probably as a result of this similarity, the plant was named *Centaurea* by Linnaeus. *C. tchihatcheffii* is vernacular named as "Yanardöner", meaning "iridescent flower" because of its attractive pink red flowers with dark pinkish red marginal florets taking an iridescent shimmer in the sun. The plant is also called "Golbasi cornflower", "bride button", "tomb cornflower", "red cornflower" or "Golbasi love flower" in the region (Ekim, 1994).

The species was first introduced to the scientific world in 1854 by Fischer and Meyer. The sample type was picked up by the Russian scientist Pierre de Tchihatchedd in 1848 at the Mehmet village near Afyonkarahisar. For this reason, the plant was called *C. tchihatcheffii* reffering to this researcher. The taxonomic evaluation was made 6 years after the plant was picked, and in 1854 it took its place as a new *Centaurea* species in science. This species was cited in 1875 in the 3rd edition of *Flora Orientalis* as *Melanoma tchihatcheffii* (Fisch. and Mey.) Boiss, and as *Centaurea purpureiradiata* Hub.-Mor. in 1967 by Huber-Morath. The last two species names have been eliminated as it was Wagenitz, who made the revision of the *Centaurea* genus and established the correct name according to the rule of priority and maintained the name *C. tchihatcheffii* species which had been firstly published. *C. tchihatcheffii* was picked from a different locality (Ankara-Golbasi-Mogan Lake) by Prof. Dr. Kamil Karamanoglu and compared with the type sample of the *C. purpureiradiata* species. In the following years the plant was also picked by Brown and Kotte from the same region. This plant is no longer found in the area where it was first discovered, in Afyonkarahisar. It is known that the plant today grows only on a very limited scale around Ankara-Golbasi-Mogan Lake (Erik et al., 2005; Tarikahya, 2005).

The profile of *C. tchihatcheffii* as defined by Erik et al. (2005) is described below. Profile: B4 Ankara: Golbasi, 18 v 1959, Karamanoglu (type of *C. purpureiradiata*); 900 m, E. S. Brown 316; Ankara to Haymana, Kotte 1109. B4 Ankara: Golbasi, Hacılar village, Forest Recreation, Step, 850 m, Erik 6330, B. Mutlu; State Opera and Ballet area, fallow land, 830 m, 10.6.2004, Erik 6360, B. Mutlu, B. Tarıkahya. *C. tchihatcheffi*, a highly attractive plant with multicoloured flowers, has a high potential to be evaluated as a decoration plant.

PREVIOUS RESEARCHES OF *C. TCHIHATCHEFFII*

In most of the studies of *C. tchihatcheffi*, which struggles to survive in a very limited area in Ankara-Golbasi, primary attention has been paid to the importance of the plant in the flora of Turkey and its relationship with other plants. It has been studied in terms of biodiversity and ecological systems, and consequently it has been concluded that this plant will become extinct if precautions are not taken.

The morphologic, taxonomic and ecologic features have been defined in the studies of the species. The height of the annual *C. tchihatcheffii* is 20 − 50 cm and the stem which carries a capitulum is perpendicular (Figure 1). A woody tap root system is observed. Generally, the feathery leaves show differences according to the places in which they exist. Each capitulum in the plant is generally 20 – 25 × 10 – 15 mm. It bears pink, purple, bright red flowers. The involucre is 10 − 15 × 11 − 15 mm and has a broad campanulate shape. The length of the appendages which exist on the involucre is 1, 5 - 3 mm and have 6 - 23 white papillomas on their edges. The pappus which is calix originated, is largely found on the point of the achene and is fawn coloured. It is 1 – 3 mm and is fleecy. The length of the achene is 3, 5 - 4(5) mm, and the hilum is very explicit (Figure 2). Anther tube is rose-purple. The peculiar form of the funnel-shaped marginal flowers with crenate margin is unique to this genus. The anther tube is provided with glands at the tip of the appendages, a character so far only known in this species. The flowering time is the end of April and beginning of May (Agababian, 1990; Kaya and Genc, 2002; Cakırlar et al., 2005a; Erik et al., 2006; Ozel et al., 2006a, Uzunhisarcıklı et al., 2007).

The necessary definitions have been made by analysing the anatomic features of the root, stem, leaves and seed shell of the species (Kaya and Genc, 2002; Cakırlar et al., 2005a, 2006; Cölgecen and Büyükkartal, 2009).

In very few studies *C. tchihatcheffii* has been determined to have antiviral, antibacterial and antifungal properties (Koca and Özcelik, 2009; Koca et al., 2009). In the studies in which the population dynamics, natural threats and effects and the life and reproduction success of *C. tchihatcheffii* have been evaluated, it has been determined that the species shows weed behavior, high population density (~18.5 − 63.2 individuals/0.5 m²), a permanent seed bank including many living seeds (~20,000 seed/ m²) and a rapid development under appropriate climate conditions. The seed production has been realized by itself or by cross pollination with general pollinators (Cakarogulları, 2005). It has been noticed that there is no natural threat that might prevent the permanency of the *Centaurea* population. It has also been determined that the most serious threats are the results of human activity, like corn cultivation, use of intense herbicides, intensive construction, the plant's potential as a decoration plant and uncontrolled plucking and sale as a cut flower (Vural and Adıgüzel, 2001; Arif et al., 2004; Bosgelmez et al., 2005a,b; 2006; Tan and Vural, 2007).

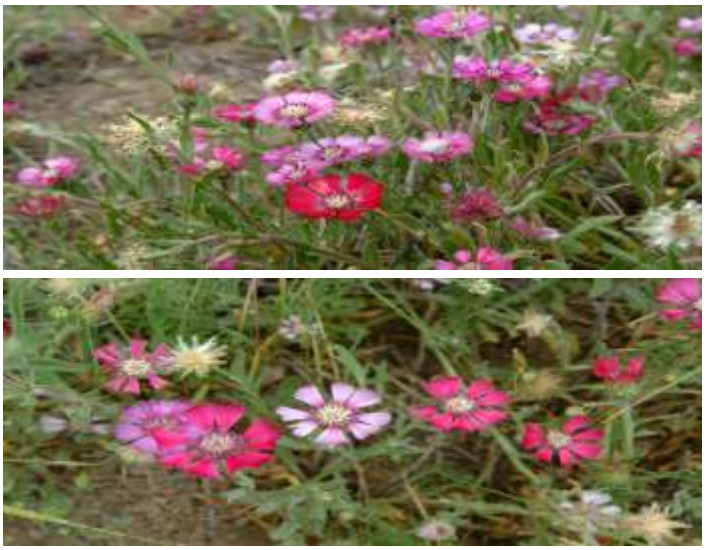

Figure 1. Two views of *C. tchihatcheffii* and flower structures *in situ.*

Figure 2. The achene type fruit of *C. tchihatcheffii* (A) Pappus (B) Hilum.

During the studies made on the fertilization biology of the *C. tchihatcheffii*, it has been ascertained that the species is composed of 10 homologous chromosome pairs at somatic metaphase stage and that the somatic chromosome number is 2n=2x=20. The basic chromosome number has been determined to be x=10, and this is an indicator of the decreasing disploid in this species (Gömürgen and Adıgüzel, 2001; Gömürgen, 2006; Martin et al., 2009). It has been determined that the *C. tchihatcheffii* pollens have all the features of the *Cyanus* group and are evolutionarily more developed than the pollens of the other species (Pehlivan, 1994, 1995; Dogan and Özmen, 2006; Celik et al., 2007).

It has been determined that while explaining the reasons for endemism, we can take into account the soil features in which the plant grows and the nutrient content of the plant. The areas in which *C. tchihatcheffii* grows are heavy structured soils which include a high proportion of clay. Because of insufficient rain the saturation values of the soil have increased and high pH values (8.03 − 8.88) have been reached in the areas where the plant grows. It has been determined that the nitrogen and phosphorus content is very low or low, Cu level is sufficient, Zn level is low, Mn level is very low or low, Fe level is medium or more while B contents are abundant or sufficient where the plant shows natural deployment (Özcan et al., 2005; Dengiz et al., 2006).

PROPAGATION OF *C. TCHIHATCHEFFII*

Many plant species are lost every year. The main point in protecting these species is to reproduce them effectively. The studies on the propagation of endemic plants are limited. This is also true for the *Centaurea* species. Only limited number of studies on propagation of *Centaurea* species including *C. tchihatcheffii* are available.

Previous studies mainly focused on early flowering, increasing the number of flowers and suitable sowing period for *Centaurea montana* (Cox, 1988) and *Centaurea moschata* var*. imperials* (Selaru and Draghici, 1989). Callihan et al. (1993) and Davis et al. (1993) conducted studies on the seed storage times for *Centaurea soistitialis* and *Centaurea masculosa* respectively.

Certain tissue culture methods have been applied in the protection of the endangered species and in the production of the *Centaurea* species which are difficult to reproduce. Tissue culture studies conducted in the *Centaurea junaniana* (Hammat and Evans, 1985), *Centaurea paui* (Cuenca et al., 1998), *Centaurea cyanus* (Kakegawa et al., 1991; Tanimoto and Ishioka, 1991), *Centaurea macrocephala* (Hosoki and Kimura, 1997; Takashi and Daisuke, 1997), *Centaurea spacchii* (Cuenco and Marco, 2000), *Centaurea rupestris* (Perica, 2003), *Centaurea zeybekii* (Kurt and Erdag, 2009) and *Centaurea ultreiae* (Mallon et al., 2010), the sterilization applications, rooting and shoot formation mediums were studied. However, none among these studied optimised conditions suitable for propagation of the *Centaurea* species.

Özel (2002) found that the best result in terms of seed surface sterilization were achieved with 50% bleach in 30 min and with 60% bleach in 10 − 20 − 30 min at 93.33%

rate. The best result for the surface sterilization of the unmatured embryos of the *C. tchihatcheffii* were achieved after 30 min application of 50% commercial bleach. The best result and the highest value of the surface sterilization of the explants obtained from plants growing outside was achieved with double sterilization using 30 and 40% commercial bleach for 5 min or with single sterilization using 35% commercial bleach for 15 min.

In another research conducted by Tipirdamaz et al. (2006), the seeds of *C. tchihatcheffii* were cultured in the MS medium containing 6 different concentrations of agar in range of 6 - 9 g l^{-1} for germination. Also, the shoot explants taken from the *in vitro* seedlings developed were incubated in MS and ½ MS media containing 9 g l^{-1} agar, supplanted with 1 mg l^{-1} GA_3 + 0.225 mg l^{-1} BAP. The results showed 40.7% shoot regeneration frequency. In ½ MS medium 42.3% of the explants formed shoot, and 38.4% continued to develop by themselves with 2.27 shoots per explant on both media.

Özel et al. (2006a), who worked on the micropropagation and shoot regeneration used immature zygotic embryos and stem nodes of *C. tchihatcheffii*. They observed no micropropagation from stem nodes. The immature zygotic embryos showed the highest shoot regeneration in the MS medium including 1 mg kinetin and 0.25 mg l^{-1} NAA. The best rooting was achieved on MS medium supplemented with 1 mg l^{-1} IBA.

Taking into account that the optimizing studies of the tissue culture conditions are ongoing, the most appropriate reproduction method is seed propagation. Seeds of *C. tchihatcheffii* have appropriate structures for dispersion and consist of fruits called achene.

It is found that the *C. tchihatcheffii* reproduces with seeds in nature, but when the seeds are germinated using classic methods the results are negative. In researches in which effects of pH value of the sowing medium, different degrees of temperature, gibberellin applications, and effects of photoperiod which are the important factors of germination have been studied, signs of germination have not been detected. The seeds of the species show high dormancy and are germinated with difficulty. It has been noted that the dormancy period of *C. tchihatcheffii* seeds kept under laboratory conditions (20°C, 50 - 60% humidity) lasts for 9 months. It has also been ascertained that the seed shell is not impermeable for water and gas and that results in dormancy of embryo (Özel, 2002; Cakırlar et al., 2005b; Özel et al., 2006a).

In *C. tchihatcheffii*, seeds which are picked from nature and incubated for 12 h sown in different media at 20±1/15±1°C morning/night temperature, 50% humidity, only 1 − 20% germination has been observed. The germination rates of the seeds, the pappuses of which have been cut or not cut, germinated in the 14 h light period and 18±2/14±2°C morning/night temperatures, are at very low rates (32 − 36%) (Cakırlar et al., 2006).

It has been determined that the *C. tchihatcheffii* seeds could not germinate at high temperature levels; that the germination ability is not lost when pre-cooling applications are administered and the germination is 10%, such that the germination is higher in seeds which endured a longer domancy period after being collected; that germination in the soil + perlite mixture^{-1} is completed in 7 − 10 days in seeds kept for 9 months after collection and the germination rate reaches 90%, and such that the flowering phase is completed within 50 − 55 days. Also, it has been ascertained that the pH level also has an effect on seed germination (Cakırlar et al., 2005b, 2006).

As to the germination of *C. tchihatcheffii* seeds, the effects of different pH levels in the germination medium of the seeds, their being kept in water and GA_3 solutions as well as being kept in a stratification medium before sowing have been studied. Parallel with the other researchers' germination rates have been shown to be low. Nevertheless, the germination rates are higher in seeds kept in a highly concentrated GA_3 solution (100 ppm) for 24 h and stratified for 120 − 150 days (in turn 45 − 46% and 48 − 76%) before sowing. Also, it is observed that the 6.5 and 7.5 pH values produced high results in the germination of *C. tchihatcheffii* seeds (35 − 76% and 35 − 63% respectively), and that seed germination is prevented when the alkalinity of the soil increases (Günöz, 2008; Okay and Günöz, 2009).

In the studies conducted to investigate the possibility of propagation the plant with vegetative methods, the 8 − 10 cm young shoots collected up from nature were kept in IBA and NAA solutions at different doses (0 − 500 and 1000 ppm) for different periods (0, 5 − 10 and 15 min), rooted in flowerpots including sterile sand and the rooted plants were planted in flowerpots. It was observed that root formation started from the 10th day and at the end of the 2nd week was completed except control application. The best rooting, root length and normal flowering was acquired in the 500 ppm IBA and during the 10 min application (Özel, 2002; Özel et al., 2006b).

CONCLUSION AND SUGGESTIONS

Golbasi-Mogan lake, which is one of the "Zero Annihilation Areas", hosts the *C. tchihatcheffii* plant, which is a critically endangered endemic plant species. There is no natural limitation on population persistence. Besides physiological factors, the plant is also threatened by human intervention. It is suggested that the natural areas should be protected for the continuity and protection of the plant by encouraging studies on different aspects limiting the propagation of the *C. tchihatcheffii* for use in routine production of the plant. Besides, emphasising optimization of propagation conditions through tissue culture, efforts should also be made to propagate the plant through seed, which may become the most

effective method for propagation of *C. tchihatcheffii* and conservation of germplasm of this critically endangered plant.

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