

Optimization of tissue culture for multiplication of valuable TK genotypes

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ABSTRACT

The species *Taraxacum kok-saghyz* (TK) possesses reproductive characteristics that are challenging, as we work toward developing a predictable, high rubber yielding field crop; there is considerable variability in flowering time between individual TK plants, it is self-incompatible (can't self pollinate) and some plants are male sterile. Tissue culture is a way to conserve and multiply our most valuable germplasm e.g. plants with modified ploidy levels (mostly tetraploids), genome edited plants and plants with unique characteristics (high rubber producers, herbicide resistant, early flowering, early germination, etc.).

TK has shown an exceptional and rapid response to tissue culture. By addition of growth regulators to culture media, shoot cultures can be obtained from leaf tissue and flower organs within three to five weeks both in diploid or tetraploid accessions. Leaf tissue produces well formed plantlets and slightly faster than ovaries, which also produce abundant callus which is of interest for research applications.

INTRODUCTION

Tissue culture is an effective technique that allows mass cloning of plants of interest, for both breeding and research. The optimization of this technique for TK is key to improve, multiply and conserve germplasm for current and subsequent projects. Germplasm can be conserved indefinitely. Results of tissue culture on leaves and flower organs of diploid and tetraploid TK plants are presented.

MATERIALS AND METHODS

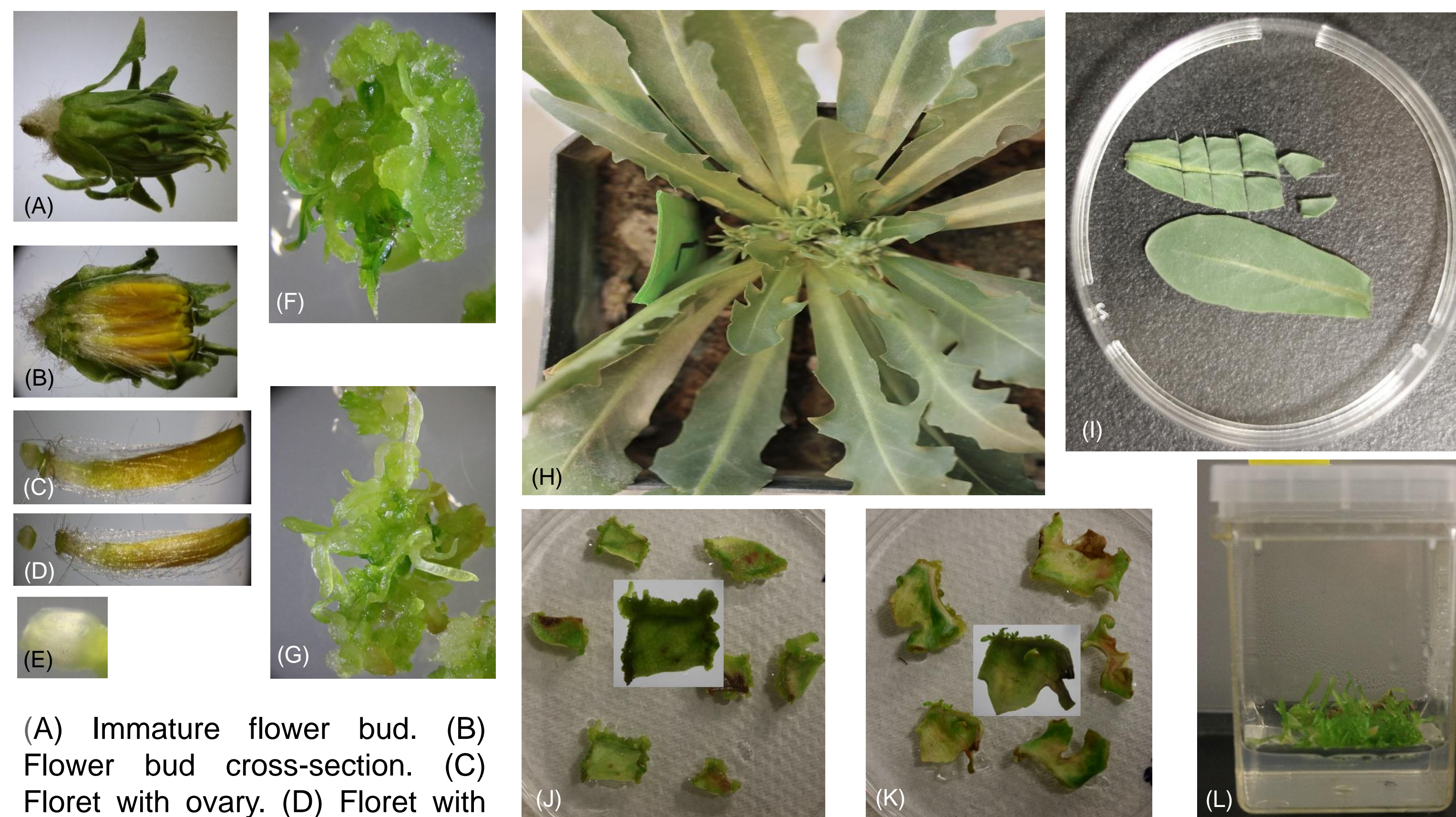
Leaves and flowers were collected from several accessions of diploid and tetraploid TK – immature flower buds and/or young expanding leaf tissue. The tissue was sterilized in a 5% bleach solution for 15 minutes and rinsed with sterile water 5X.

One young flower bud from each plant was cut open, the ovary was excised from the floret and placed on media – the remaining (floret) organs can also be used. Two young, expanding leaves were cut in two pieces down the midrib, then cut crosswise into approximately 1 cm pieces before placing on culture media. The culture medium was composed of a Murashige and Skoog (MS) mix with Gamborg B5 vitamins, supplemented with a combination of the growth hormones BA (6-Benzyladenine) 0.5 mg/L and NAA (1-Naphthaleneacetic acid) 0.1mg/L or BAP (6-Benzylaminopurine) 1mg/L and IAA (Indole-3-acetic acid) 0.2 mg/L. BA and BAP are cytokinins and NAA are auxins. Tissue was also placed on medium containing 0.5mg/L BA and 20mg/L 2,4-D. Gelrite was used a gelling agent. The cultures were placed at 23 °C under a light regime of 16h.

RESULTS

Clonal plants have been successfully generated through tissue culture. Healthy green tissue was recovered from media supplemented with NAA+BA or BAP + IAA while the treatment with 2,4-D produced only callus, which may be embryogenic and some possibly haploid (from flower parts). Shoot recovery rates vary greatly from explant to explant, the age of the tissue being the most probable source of variation. Styles and stigmas produced callus but no shoots have yet been recovered.

Ovaries are very responsive producing callus in ~2 weeks with plantlets emerging from the callus. Explants are ready for shoot elongation and root induction in 4-5 weeks. In leaf culture, shoots begin forming ~2 weeks after culture, and explants are ready for shoot elongation and root induction in 3-5 weeks. While culture to plant time is faster with leaf tissue there are also issues such as a high incidence of contamination and the number of explants recovered from leaf tissue are far fewer that can be obtained with ovary cultures.



(A) Immature flower bud. (B) Flower bud cross-section. (C) Floret with ovary. (D) Floret with ovary removed. (E) Ovary in culture. (F) Shoots forming on diploid ovary tissue after 20 days. (G) Shoots forming on tetraploid ovary tissue after 20 days.

(H) Young TK plant with immature flower buds and young expanding leaves. (I) Young expanding leaves. (J) Diploid TK leaf pieces after 16 days in culture. (K) Tetraploid TK leaf pieces after 16 days culture. (L) Shoots in elongation media.

Remarkably, the responsiveness of ovary and leaf tissues is conserved in tetraploid TK accessions. It allows us to apply the same procedures developed for diploid TK accessions to tetraploid TK. Again, a key point for the success of tissue culture is the age of the tissue used and age appears to be inversely proportional to responsiveness, as common in other systems. Thus, organs from immature flowers at the bottom-center of the rosette (i.e. flowers before petiole-elongation, also known as bolting) were more responsive and successful in generating healthy explants.

DISCUSSION

The ease with which *Taraxacum kok-saghyz* clonal tissue can be generated makes it easy to adopt into a germplasm-development program. The responsiveness of the tissue is comparable to that of plant model species such as *Arabidopsis thaliana*, tobacco and petunia, and seems to be independent of ploidy level. Tissue culture of many species in the Compositae (Asteraceae) family, such as, sunflower, safflower and lettuce, is challenging (Záveský 2010). Reports on chicory (Vasseur et al. 1995) though, suggest a more positive response, as seen in TK.

It is well known that the age of the tissue is highly influential in the success of tissue culture; however, in the case of TK, size does not necessarily correlate with the physiological/ontogenetical age of the tissue. Thus, a careful tracking of chronological age of tissue (leaves and flowers) is needed. The development of a protocol to obtain embryogenic tissue from leaves, ovaries and other flower parts, as well as haploid cultures from anthers, would be greatly beneficial.

CONCLUSIONS

The results on the optimization of tissue culture for TK suggest that it is an effective technology for massive multiplication of relevant genotypes. Ovary and leaf culture are the most responsive organs, irrespective of ploidy level, and healthy explants are obtained with a culture medium of MS+Gamborg+NAA+BA. Tissue culture from ovaries would allow us to clone relevant genotypes of similar ontogenetical age and in a high-throughput fashion (at least 50 explants per flower). Cloning from leaf tissue allows for plants to be cloned irrespective of the season. The age of the tissue is very relevant, and careful tracking of the age of organs is needed for future endeavors.

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