# Cryoconservation of hop pollen

**[1] Svoboda P., [2] Faltus M., [1] Nesvadba V., [1] Charvátová J.**

[1]Research Institute of Crop Production, Prague, Czech Republic

[2]Hop Research Institute, Co., Ltd., Žatec, Czech Republic

## Introduction

The hop plant, *Humulus lupulus*, L., is a dioecious perennial species, and only female clones are used for beer brewing. Hops are used to impart bitterness, flavour and preservation properties to modern beers.

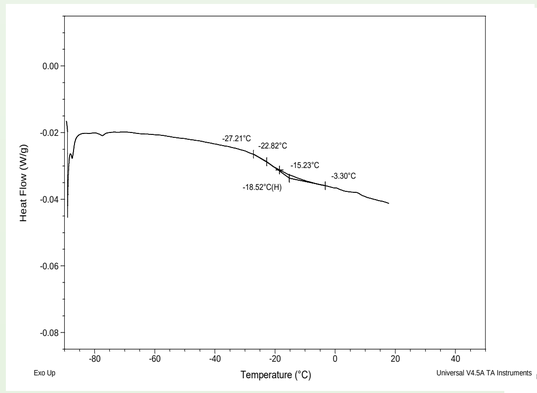
Storage of pollen not only effectively overcomes the obstacles in crossing of parents flowering at different times and in different geographical locations, but also is an effective means to preserve the genetic diversity of plants (2019).

## Material and methods

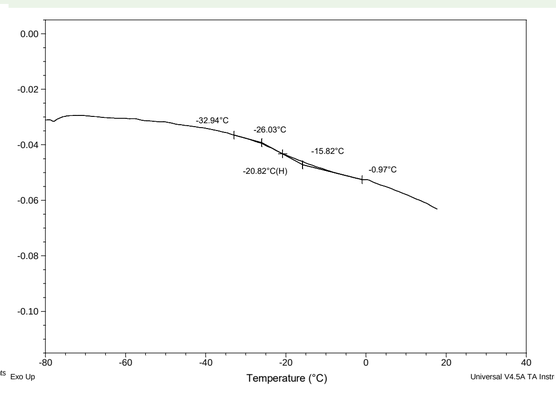
Multi-branched panicles of selected individuals of male hop clones (12/00, 12/06, 19/07) were collected in July 2019. Hop pollen grains were spontaneously released from panicles and collected into plastic test tubes in laboratory conditions. Thermal analysis of hop pollen was performed using differential scanning calorimeter (DSC) Q2000 (TA Instruments, USA) with refrigerated cooling system (RCS) in the temperature range from -90 to +20°C. The cooling rate was 10°C /min. Temperature-modulated DSC method was used during warming cycle with warming rate of 1°C/min. Temperature modulation was performed at 1°C amplitude of modulation and 60 second period. Aluminium, hermetically sealed pans were used, sample size ranged from 5 to 20 mg and the purge gas was nitrogen.

## Results

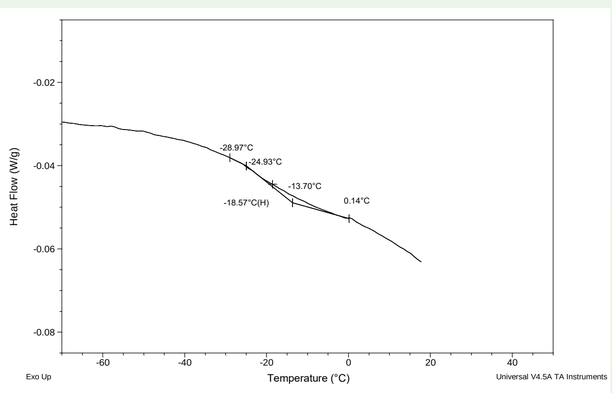
No significant endothermic or exothermic events were detected in all genotypes tested. On the other hand, the glass transitions were detected in all samples during warming cycles, and they ranged from -35 °C to -10 °C. The half-height of sample glass temperature was -18 °C, -20 °C and -28 °C in clones 19/07, 12/06 and 12/00, respectively. The determined thermal characteristics of all hop clones tested make their cryopreservation possible. Resulting thermal properties were influenced mostly by water content and, conversely, the effect of variety and plant species was not significant; the dependence of the studied parameters was a function of the water content regardless of the tested genotype. It was found that when the water content falls below 0.27 g of water per g of dry matter, water does not crystallize and at the same time, the presence of a glass transition has always been demonstrated.




Thermal analysis of hop pollen, clone 19/07. The glass transition temperature is expressed as a half-height (H) of the s-shape curve.




Thermal analysis of hop pollen, clone 12/06. The glass transition temperature is expressed as a half-height (H) of the s-shape curve.




Thermal analysis of hop pollen, clone 12/00. The glass transition temperature is expressed as a half-height (H) of the s-shape curve.

## Conclusion

The thermal analysis of all hop clones tested detected no freezable water content presence. The occurrence of the glass transition guarantees conditions for successful cryopreservation of pollen samples.

**Acknowledgement**

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**Literature**

Changes of pollen viability of ornamental plants after long-term preservation in a cryopreservation pollen bank. Cryobiology 89 (2019) 14–20