

# A REVIEW OF PROPAGATION TECHNIQUES AND ISOTHIOCYANATES CONTENT IN WASABI (*WASABIA JAPONICA* MATSUM.)

Věra Kofránková<sup>1</sup>, Martin Koudela<sup>1</sup>

<sup>1</sup>Department of horticulture, Faculty of Agrobiological Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha 6-Suchbát, Czech Republic

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## Abstract

Japanese horseradish (*Wasabia japonica* Matsumura) is a perennial plant originated from Japan, but is currently grown in many other countries across the world. Wasabi contains important substances isothiocyanates (ITCs) which have potential positive influence on human health. Wasabi extract is also eventual a plant protection product. Therefore, using these positive features and obtaining an effective multiplication of this crop is an important element. This work focuses on methods of Wasabi propagation especially with the use of in vitro culture and the possible influence of the content of ITCs-allyl isothiocyanate (AITC) on important substances in Wasabi.

Keywords: *Wasabia japonica*, japanese horseradish, propagation, in vitro, isothiocyanates, allyl isothiocyanate, phytohormones

## INTRODUCTION

Wasabi (*Wasabia japonica* Miq. Matsumura, syn. *Eutrema wasabi* (Siebold) Maxim.) is a perennial plant of the *Brassicaceae* family. It is native to Japan where it grows wild, mainly along the mountain streams (Chadwick *et al.*, 1993; Rubatzky and Yamaguchi, 1997). Due to suitable soil and climate conditions, growing of Wasabi spread in the early 1980's to Tachikawa, Columbia, Canada, Korea, Thailand and USA (Douglass and Follet, 1992), to New Zealand (Martin and Deo, 2000; Sultana *et al.*, 2002) as well as to Australia and Tasmania (Sparrow, 2006).

*Wasabia japonica*, also called 'Japanese horseradish' is valued for its culinary use in

traditional Japanese cuisine. However, research results suggest other possible uses of this crop in plant protection and in human medicine. Wasabi has a strong antimicrobial (Depree *et al.*, 1999; Delaquis and Mazza, 1995; Khan *et al.*, 2006), anti-inflammatory (Yoshida *et al.*, 2015; Uto *et al.*, 2005) and antibacterial effect (Shin *et al.*, 2004). Many authors recorded positive medicinal effects of Wasabi-extracted substances. Weil *et al.* (2005) reports an ability of Wasabi extracts to inhibit tumor cells. According to other studies, Wasabi leafstalk extract stimulates bones calcification and it might be used in osteoporosis prevention (Suzuki *et al.*, 1997; Suzuki and Masayoshi, 2004; Yamaguchi, 2016). 6-methylsulfinylhexyl isothiocyanate isolated from Wasabi has positive

antioxidant effects in diabetes renal dysfunction (Fukuchi *et al.*, 2004). The research also shows a possible use of transgenesis of a wasabi gene; in potatoes, the wasabi gene has a positive role in partial resistance to *Botrytis cinerea* (gray mold) (Khan *et al.*, 2006).

To obtain healthy Wasabi seedlings is important both for commercial production of thickened underground parts (rhizomes) used for preparation of a wide range of Japanese cuisine dishes, and for medicinal and other research. Healthy seedlings need at least 1.5 year to reach the harvest maturity (Ehret *et al.*, 2004), yet, they are often harvested later.

## PROPAGATION

Wasabi may be propagated both in generative and vegetative ways. Seeds are formed in the first half of summer (from the onset of flowering to seed maturity it lasts about 2 months); seed viability depends on the temperature course over the flowering period (Adachi, 1987).

Fresh seeds are dormant and the dormancy lasts for 8 months after their harvest. It is possible to remove dormancy either at low-temperature stratification using gibberellic acid (Palmer, 1990) or by seed coat scarification (Dedree *et al.*, 1998; Nakamura and Sathiyamoorthy, 1990). Wasabi seeds belong to the group of so-called recalcitrant seeds (i.e. do not survive drying and freezing), and thus they are difficult to store (1 year at maximum). Moreover, they cannot be stored at low relative humidity (Gross *et al.*, 2016), as they are sensitive to desiccation (Matsumoto *et al.*, 1994). Gross *et al.* (2016) thus recommend storage at high relative humidity of 92–98 %, which may, however, cause some other problems because of a higher risk of microbial contamination.

Freezing seeds and the storage in temperatures below 0 °C is difficult because of high seed water content. The only possible storage method is cryopreservation. It is necessary to induce so-called vitrification, i.e. transformation of seed water to glass non-crystalline amorphous solid without formation of ice crystals in the cells during freezing. It requires fast cooling of small samples – suitable are extracted embryos or embryonic axes (Pammenter and Berjak, 1999). For its financial and time demands, this technique is suitable to store the genetic material in the bene bank, not for common seed storage (Engelmann, 2004). From the above-mentioned theses, and especially with respect to a fast decrease of germination ability of Wasabi seeds (Depree *et al.*, 1999), it results that Wasabi plants are mostly

propagated using vegetative methods (Palmer, 1990; Rubatzky and Yamaguchi, 1997).

A common way of vegetative propagation of Wasabi is using side shoots that are obtained from plants at rhizomes harvest (Rubatzky and Yamaguchi, 1997). At harvest, the plants are extracted from soil manually and the side shoots that are healthy and strong enough can be immediately used to establish new crop. Side shoots that are removed of the mother plant should have at least 4–5 leaves, 4 cm in height and be free of symptoms of diseases. If the shoots are big enough, they may be planted at the land immediately after they were removed from the parent plant. However, if they are too small, it is recommended to let them grow before planting. Depending on the variety and size of the mother plant, it may provide up to 20 side shoots (Miles and Chadwick, 2008).

The most important difficulty of Wasabi vegetative propagation is the health of seedlings. Thus, it is recommended to use the side shoots only once in the subsequent vegetation. To ensure the best health conditions of the plants, the propagated material should be taken from meristem cultures (Rodríguez and Punja, 2009).

Wasabi plants are sensitive to a wide range of pathogens, which is a major problem in the propagated culture. Vascular blackening of rhizomes causes a significant deterioration of the consumed part of the plant. In past, the authors reported *Phoma wasabiae*, *Erwinia* ssp. as the causal agent of blackening (Ehret *et al.*, 2004; Lo and Wang, 2000; Martin and Deo, 2000). Yet, Rodríguez and Punja (2009) contradicted that, determining the agent of blackening as *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*). The previous results may be related to incorrect determination of pathogen that was present only on the rhizome surface. Pathogens *Phoma wasabiae* and *Plasmidiophora brassicaea* (Adachi 1987; Chadwick *et al.*, 1993) are present at Wasabi plants but less than the above-mentioned *Pcc*.

To conclude, it is obvious that traditional ways of both generative and vegetative propagation of Wasabi are related with phytopathological and physiological problems; it is thus important to use certified plants from meristem cultures (Rodríguez and Punja, 2009).

## In vitro

The basic material used for Wasabi in vitro propagation are dormant buds of mature rhizomes (Hung *et al.*, 2006), axial buds (Rodríguez, 2010) or apical meristems (Matsumoto *et al.*, 1994).

The standard cultivation medium used for in vitro propagation is the MS medium according to Murashige and Skoog (1962). Ichi *et al.* (1986) recommended the LS medium (Linsmaier and Skoog medium) with addition of gellan gum for Wasabi cultivation. Hung *et al.* (2006) compared liquid and gelled media and different concentrations of the MS medium:  $\frac{1}{4}$  MS;  $\frac{1}{2}$  MS; MS. Between  $\frac{1}{2}$  MS and full-strength MS no statistically significant effect on the Wasabi plants cultivation was observed.  $\frac{1}{4}$  MS medium was reported as the least suitable for root proliferation as compared to the full-strength liquid medium that was the most effective after four weeks of culture. These results are in compliance with the study of Park *et al.* (2007). The authors choose both concentrations of MS medium for their experiments:  $\frac{1}{2}$  MS medium (Matsumoto *et al.*, 1994; Matsumoto *et al.*, 2013; Hung and Johnson, 2008), full-strength medium (Hoang *et al.*, 2017).

In the experiments of Hung *et al.* (2006), the most effective media to reach the maximum number of shoots were half- and full-strength MS liquid media compared to semi-solid medium or quarter-strength MS liquid medium. Hung *et al.* (2006) ascribe the positive results of the liquid medium in the *Wasabia japonica* culture to better accessibility of nutrients, oxygen and saccharose. After 6 weeks of culture the difference in shoot proliferation was apparent;  $\frac{1}{2}$  MS liquid medium produced more shoots than the full-strength liquid medium. Moreover, after 6 weeks of culture, the full strength medium plants showed symptoms of hyperhydricity, whereas in the  $\frac{1}{2}$  MS and  $\frac{1}{4}$  MS liquid media these symptoms were not observed (Hung *et al.*, 2006). Hence, for Wasabi in vitro cultivation it is recommended to use the full-strength MS medium at the beginning of culture and after 4 weeks pass to  $\frac{1}{2}$  MS medium.

The most suitable gelling agent for Wasabi cultivation in in vitro conditions is then Gelrite due to its purity and consistent quality that cannot be reached with agar (Huang *et al.*, 1995). Gelrite is suitable for plant tissues as well as for plant regeneration; in test, it gave better results in 0.2 % concentration than 0.8 % agar. At the recommended concentration, the solidity of Gelrite is lower compared to agar. Higher concentration helps obtain more solid medium; however, the higher the gelling agent concentration, the lower the proliferation intensity. Using 0.6 % Gelrite the proliferation was lower, yet not eliminated. For callus cultures, the recommended Gelrite concentration is 0.15 % (Huang *et al.*, 1995). For shoot multiplication

Hung *et al.* (2006) suggests Gelrite concentration of 0.7 % as compared to Huang *et al.* (1995) who recommend 0.2 % Gelrite.

### EFFECT OF CYTOKININS AND AUXINS ON SHOOT PROLIFERATION.

Optimum shoot numbers were obtained at cytokinins concentrations as follows: 0.5–10.0 mM N<sup>6</sup>-benzyladenine (BA), 0.5–10.0 mM thidiazuron (TDZ), 5.0–50.0 mM kinetin, and 10.0 mM zeatin. The highest values of shoot numbers were obtained in the full-strength MS medium with kinetin (10.0 mM) and BA (5.0 mM) (Hung *et al.*, 2006). Hosokawa *et al.* (1999) reported the highest number of shoots, in the medium with BA. Their experiment showed as the most optimal concentration 1mg/l BA. Higher concentrations (5; 10 mg/l BA) provided lower values. On the other hand, lower concentrations were used for micropropagation by Hoang *et al.* (2017) and Matsumoto *et al.* (2013), 0.5 mg/l BA and 0.1 mg/l BA, respectively.

*Wasabia japonica* forms roots in media also without using hormones; however, at the optimal concentration of auxins 3-indole butyric acid (IBA), 1-naphthaleneacetic (NAA) and indole-3-acetic acid (IAA) the roots are formed sooner. The best concentration for root formation was IBA at 0.5 mM – the roots were formed in 1 week. Compared to that, NAA at 10.0 mM strongly inhibited root growth (Hung *et al.*, 2006).

### AITC CONTENT OF WASABI

Characteristic taste (acridity) of Wasabi is given by a set of substances – isothiocyanates (ITCs); they are formed by a hydrolysis of precursors (glucosinolates) – substances contained in plants of the whole *Brassicaceae* family (Chadwick, 1993; Sultana *et al.*, 2002; Sultana *et al.*, 2003b). Horseradish, which has a similar taste, also contains these substances, but in lower concentrations than Wasabi (Sultana *et al.*, 2003a). Volatile ITCs release allyl isothiocyanate (AITC), which is the main substance responsible for anti-microbial characteristics of Wasabi (Sultana *et al.*, 2000; Sultana *et al.*, 2002). AITC contributes to 89–94 % ITC groups in Wasabi plants; the highest concentration of these substances is in plant rhizomes, which is also the most used Wasabi plant part (Sultana *et al.*, 2000).

Increase of AITC compound in Wasabi crop would have a positive impact in agricultural, pharmaceutical and industrial usage (Hung and Johnson, 2008).

The content of AITC in different parts of Wasabi plants may be influenced by agrotechnical measures – fertilization (Sultana *et al.*, 2002), storage (Sultana *et al.*, 2003b) as well as by selected factors of in vitro culture conditions (Hung and Johnson, 2008).

Proper fertilization can influence the overall Wasabi yield during cultivation. Sultana *et al.* (2002) suggest an important role of sulphur in Wasabi fertilization. The highest yields of rhizomes were obtained at stands fertilized with ammonium sulphate (increase up to 74 %). Rivelli *et al.* (2016) comply with the previous results; yet, they outline the importance of genetic predisposition for the AITC content in Wasabi. Manure positively influenced the content of AITC in Wasabi leaves; however, as leaves are a less used plant part,

nitrogen fertilization with sulfur amendment is recommended (Sultana *et al.*, 2002).

To preserve the ITC values during storage, Sultana *et al.* (2003b) recommend storage at temperatures –10 °C, –40 °C, –80 °C. If the rhizomes are stored in temperatures closer to 0 °C ITC values significantly drop.

It is generally recommended to grow Wasabi in a shaded culture. The present research has revealed that the most optimal rate of shading is around 10 %. Shading at a rate of 30–70 % results in a negative impact on the weight of the aboveground plant parts and thus yield (Lee *et al.*, 2008). An interesting finding was reported using Wasabi plant ionization in in vitro culture, which resulted in a positive impact on the AITC concentration (Hung and Johnson, 2008).

## CONCLUSION

History of Wasabi cultivation is closely related with Japan where it originates and is planted till today. For its highly valued rhizomes, the growers in other parts of the world try to cultivate it, some of them successfully. Wasabi is also important for research due to the compounds it contains, mainly ITC – AITC. As the results of the present research suggest, these compounds have anti-microbial potential and may be used for medicinal purposes. It is necessary to better understand how ITC accumulates in plants and the ways to affect it. The authors agree that ITCs accumulation may be influenced by N and S fertilization, however, the rates and dosage of fertilization must be determined. Another perspective technique to positively influence the AITC content in Wasabi plants is mutation. The existing research studies showed that in vitro propagation is the main way to propagate Wasabi plants. This article brings a review of culture media for Wasabi plant propagation and recovery using plant hormones.

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Contact information

Věra Kofránková: kofrankovav@af.czu.cz