Foreword

Wasabi (*Wasabia japonica*) is a brassica vegetable the rhizomes of which are used to prepare a hot-tasting condiment used in Japanese cuisine. High quality wasabi product is in strong demand by the Japanese catering industry. This demand can no longer be met locally so the potential to produce a high quality product in the cool, temperate regions of Tasmania was evaluated.

The aim of this project was to develop wasabi production in Tasmania to provide a source of the fresh product initially to the Australian market and ultimately to the East Asian market.

This report describes the development of semi-commercial production areas in Tasmania from seed imported from Japan. Propagation, site specifications, crop husbandry, market evaluation, potential for expansion of the industry and improvements in product quality are included.

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

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Acknowledgments

I would like to acknowledge the contribution made by DPIWE staff involved in the wasabi project, Michael Hart (Project Supervisor), Nani Clark (Development and Marketing Officer) and Mel Barber (Principal Investigator at commencement of project). I would also like to acknowledge the help given by DPIWE staff for field and glasshouse work and clerical assistance.

I would like to thank Ian and Diane Farquhar and Neville and Edna Williams for their hard work and perseverance in managing the field trial sites for wasabi on their properties and for the generous hospitality shown to me and visiting enthusiasts at every visit.

Abbreviations

DPIWE Department of Primary Industries, Water and Environment
TIAR Tasmanian Institute of Agricultural Research
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Executive Summary

Seed of *Wasabia japonica* variety 'Daruma' was imported from Japan in October 1997. The seed was tested for viability, storage and virus status prior to being propagated by a local nursery. Two semi-commercial sites were established in North-Eastern Tasmania by August 1998, each approximately 0.2 ha in area.

Shade house structures were erected on sheltered sites and seedlings planted in raised beds to assist drainage. A fertilizer regime was established for soil-grown wasabi and the crop irrigated by overhead sprinklers. During summer months the wasabi crop exhibited some blackened lesions on the petioles. At maturity some of this blackening appeared in the stems making them unsuitable for the premium market.

Harvest of wasabi crops began in July 2000 with yields of 10 tonnes per hectare being recorded. The harvest procedure has identified the potential for yield improvements. Mature wasabi plants from imported wasabi seed variety 'Daruma' produced two types of plants, those with stems suitable for the premium market (25%) and those with stems suitable for processing (75%).

To ensure the future production of wasabi plants with premium quality stems, mature plants exhibiting superior characteristics were selected from the field in November 1999 and isolated at one site for cross-pollination to produce a source of superior quality seed. Vegetative shoots of these plants were also taken and grown to seed bearing age in glasshouse facilities at the Department of Primary Industries, Water and Environment's Mount Pleasant Laboratories. During the harvesting procedure side shoots were retained from plants with superior characteristics and these have been used for vegetatively propagating a subsequent crop.

Wasabi seed was found to have unique storage requirements. Seed harvested from the semi-commercial plots required storage at less than 5°C and 90% humidity with adequate ventilation maintained. With the chilling requirement satisfied, germination rates of 90% were recorded.

Propagation of wasabi by tissue cultural techniques was investigated for 18 months with two laboratories involved in the experimental stage. Problems with fungal and bacterial contamination hindered progress, but recent reports indicate that clean tissue has been maintained, and shoot proliferation and subdivision have been completed successfully. Tissue culture technology has the potential to produce planting material that exhibits less genetic variability than do plants propagated from seed.

Samples of wasabi from the two semi-commercial production sites were test marketed prior to commercial sale. Eleven restaurateurs or food processors were chosen to evaluate the product and their responses were very favourable. The Tasmanian product is considered to be far superior to frozen wasabi stems imported from Japan and also to the prepared pastes and powders currently available.

One commercial distributor marketed fresh wasabi from these trial sites for the six months from August 2000 to January 2001. In addition to the wasabi stem, there is demand from the market place for wasabi leaves. These were also marketed from the trial plot.

Recommendations for the future include the introduction of new varieties from Japan and Victoria, Australia to compare with variety 'Daruma'. In addition to the differences in growth habit, comparison of volatile flavour components by chemical analysis (chromatography) indicates one component is lacking in Tasmanian wasabi compared with other varieties cited in scientific literature. This component apparently contributes to the taste 'lingering on the palate'.
The incidence of blackened lesions observed on some stems increases when the plants are under stress with a number of factors thought to contribute to these symptoms. These include boron deficiency, poor drainage and high soil temperature. Such factors may be directly responsible for the observed blackening, or they may make the plants more susceptible to fungal infection.

Glasshouse trials to confirm which factors contribute to the stem lesions, and field trials to examine the value of protective fungicide in reducing their incidence should be conducted.

Traditionally, wasabi is grown in cool, running water. Key features of this type of production are the maintenance of stable root temperature and aeration. Aquatic wasabi is considered a higher quality product on the Japanese market than is soil-grown wasabi. Areas in Tasmania suitable for semi-commercial aquatic wasabi production have been identified, as have growers keen to establish such production systems.

There would be considerable value in bringing an expert in wasabi production to Tasmania either from Japan or from Washington USA where production protocols suitable for western agricultural systems have been established. This would be beneficial to developing the local industry as there is limited literature available that addresses cultural practices. In particular, advice on aquatic husbandry would be sought.
1. Introduction

1.1 Traditional wasabi

Wasabi is a perennial, semi-aquatic herb belonging to the Brassicaceae family of plants (Figure 1). It is native to Japan where it grows alongside cool shaded streams in deciduous forests. Japanese cultural practice is to plant wasabi in streambeds modified to form gravel-filled terraces through which the water flows. Wasabi paste is used with Japanese cuisine such as sushi, sashimi and buckwheat soba noodles. The paste is prepared either freshly grated from the stem of the wasabi plant and served with high quality, premium priced food or processed to make a prepared paste in which wasabi is often combined with horseradish.

The freshly prepared paste or stem segments fetch the highest price and are only served or supplied in small portions due to their high cost. In addition to the demand for fresh stems, a major market also exists for processed product in the form of paste, puree and powder. The petioles are used for pickling and the leaves and flowers used in fresh salads.

![Figure 1. Wasabi leaves and stems (rhizomes)](image)

1.2 Potential for Australian Production

The Australian restaurant trade does not currently have access to fresh wasabi. Some restaurants import frozen stems from Japan, while others use processed product. Experimentation with a range of Asian foods by consumers is increasing the demand for traditional products, so too is the inclusion of Asian flavours to complement traditional European dishes (for example wasabi served with roast beef).
The demand for fresh wasabi on the East Asian and Japanese domestic market can no longer be met by the Japanese industry due to the progressive contraction of traditional production sites in Japan caused by pollution, urbanisation and the aging population of growers. Production figures from Japan for 1998 estimate production of fresh wasabi at 6,000 tonnes per year from an area of 730 hectares. This includes both soil-grown and aquatic wasabi, a yield average of 8 tonnes per hectare.

The initial target for Tasmanian wasabi is to replace imports onto the Australia market. This will be followed by entry to the Japanese fresh market when consistency of quality and supply is achieved. Subsequently, other Pacific rim areas including Asia and the west coast of USA will be targeted.

Tasmania's international reputation for its clean environment, potential to produce in the off-season and its well developed horticultural production infrastructure lend itself to the development of boutique industries. Studies undertaken from 1995-1996 by the Department of Primary Industry and Fisheries established that Tasmania's environment is suitable for wasabi production. Potential production sites were determined on the basis of performance of the plant in field trials.
2. Project Objectives

The aim of this project was to assist in the commercialisation of wasabi production in Tasmania through:

1. Location of plant material to enable trials to be scaled up to semi-commercial size;
2. Expansion of existing sites to allow for semi-commercial assessment of agronomic techniques and technology;
3. Market evaluation of mature stems from sites already developed;

This report addresses these objectives, the results and conclusions of the research undertaken and presents recommendations for future development of the Australian wasabi industry.
3. Methodology

3.1 Location of plant material

Field trials conducted from 1995-1996 in Tasmania concluded that *Wasabia japonica* variety 'Daruma' was best suited to Tasmanian conditions. Seed of this variety was imported from Japan through 'Magnus Kahl Seeds' in October 1997. The seed was tested for viability, storage and virus status prior to being propagated by a local nursery.

The seed was germinated in seed raising mixture at 25°C in a glasshouse and seedlings pricked out and raised in cell trays. Seedlings were transplanted to field sites under 80% shade at 3 months (Figure 2).

![Wasabi seedlings planted in shade house](image)

Approximately 60,000 seedlings were produced, sufficient to establish two semi-commercial sites each of 0.2 hectares. Seedlings were planted successively to ensure an extended harvest.

3.2 Assessment of agronomic techniques

Seedling propagation

Seed was harvested from field sites each spring and autumn during the 2 year growth cycle of the crop and stored at 1°C to satisfy the vernalisation requirement prior to germination (Palmer 1990). A series of experiments were conducted to test seed viability and storage conditions. Seed cleaning methods were also investigated.
Experiment 1. To test the viability of wasabi seed following desiccation.

Whole racemes were collected when basal pods began to dehisce and stored at 1°C for 4 weeks. One hundred grams of pods were removed from the raceme and dried at 25°C. The seed was cleaned using a 'Wintersteiger' seed separator and 'Seedburo Equipment Company' blow cleaner and stored at 1°C for 12 weeks. After 6 and 12 weeks storage respectively, seed was sown in seed raising mix and placed in a shaded glasshouse at 15°C with misted watering.

Experiment 2. To test viability of seed produced along the length of the raceme.

Seed collection was made 10 weeks after flowering when basal pods on the raceme had started to dehisce. At the time of collection the racemes varied in length from 1 to 2 metres. After 6 weeks storage at 1°C each of 5 racemes was divided into 5 sections along its length. Pods were removed from the raceme and placed in trays on moist blotting paper and incubated at either 4°C or 15°C for 10 days.

Experiment 3. To establish a commercially viable method of seed extraction.

A commercial nursery agreed to propagate wasabi from seed if DPIWE could supply clean seed ready for sowing. Seed pods were harvested in November 1999 and stored in airtight plastic bags at 1°C for 4 weeks prior to extraction from the pods. Four techniques were investigated to prepare clean seed (Figure 3):

1. Seed pods placed in a nylon mesh bag and stored under running tap water at 12°C.

2. Seed pods placed in a nylon mesh bag and held in a water bath of aerated water at 12°C.

3. Seed pods placed on a nest of sieves (2.0mm, 3.3mm and 4mm) and sieved under aerated water on a wet sieving apparatus (Precision Engineering Company, Melbourne). Seed collected in the 2.0mm sieve (Wright 1988, after Yoder 1936).

4. Seed pods placed in a hydropneumatic elutriation chamber which kept the plant material agitated by introducing air under pressure. Seed collected in the bottom of the chamber and in the 2.0mm sieve. Coarse plant debris collected in the 3.3 and 4.0mm sieves (Smucker et al. 1982).

For each of the methods described above, the treatment conditions were maintained until the seed was washed free from the pods, ready for sowing.
Experiment 4. To test the effect of ventilation and removal of pods from raceme on seed viability.

A seed storage test was conducted in June 2000. Seed pods were either left attached to the raceme (flower stalk) or removed from it in the field. Seed pods were then stored at 1°C in plastic bags with moist paper towel to maintain humidity.

Four treatments were tested:
1. Seed removed from flower stalk and stored in sealed plastic bag
2. Seed removed from flower stalk and stored in open plastic bag.
3. Seed left attached to flower stalk and stored in sealed plastic bag
4. Seed left attached to flower stalk and stored in open plastic bag.

Each of the treatment samples was stored at 1°C for 6 weeks. Forty-eight seeds from each treatment were sown in trays of seed raising mixture and placed in a shaded glasshouse at 15°C.
Experiment 5. To test the influence of gibberellic acid on seed germination.

Seed harvested in November 2000, was left attached to the racemes and stored in open plastic bags with moist paper towel at 1°C. After 6 weeks seed was placed on seed germination paper soaked in 400 ppm gibberellic acid. Fifty seeds were removed from the GA soaked paper at intervals of 4, 24, 48, 72 and 120 hours and placed on germination paper soaked in distilled water. Counts of germinated seed were made after 7 days.

Seedless propagation

In July 1999, a contract was drawn up with 'Agronico' a commercial tissue culture laboratory for micropropagation of wasabi with seedlings supplied by DPIWE. In March 2000 a contract was drawn up with a second commercial laboratory 'Brio'. In August 2000 the tissue culture laboratory at Tasmanian Institute of Agricultural Research (TIAR) was invited to become involved with micropropagation of wasabi, with plants supplied by DPIWE.

In addition to micropropagation, vegetative side shoots were taken as splits from established plants and used as replacement plants at one of the sites where waterlogging had caused significant plant loss.

When the plants at the two field sites were 15 months old, 26 plants exhibiting superior morphological characteristics were isolated to a site on a nearby property for selective cross-pollination. Vegetative side shoots were taken as splits from these plants and grown in a shaded glasshouse at DPIWE Mount Pleasant Laboratories. Seed from these selected lines were collected from both the field and glasshouse plants in November 2000 and stored at 1°C. Sowing commenced in January 2001.

Semi-commercial production

Two sites were established in North-Eastern Tasmania by August 1998 each approximately 0.2 ha in area. Shade houses providing 80% shade were erected in sheltered positions at each site.

A duplicated fertiliser trial was conducted in the early stages of seedling establishment which compared the response of seedling wasabi plants to two types of fertiliser (PIVOT 800® formulation NPKS 8:11:10:7 and DYNAMIC LIFTER®) applied at 3 rates: 630, 1260 and 1890 kg/ha. Fertilisers were applied in fully randomised split-plot design. This fertiliser trial was terminated 6 months after transplanting to avoid compromising plant productivity at the lowest application rates. Plant growth was most vigorous at 1260 kg/ha PIVOT 800® so the two sites were top-dressed to bring the trial area up to the rate equivalent to 1260 kg/ha PIVOT 800®.

The crop was top-dressed at 9 and 17 months with 400 kg/ha PIVOT 200® formulation NPKS 12:5:16:11. Nitram® (33% N) fertiliser was applied at a rate of 100 kg/ha at 3 monthly intervals.

Samples of 20 leaves were taken at 2 different stages of crop development and a complete plant tissue analysis was conducted by DPIWE Division of Environment and Planning Laboratory.

At 12 months two plots (10m x 1.5m) within the crop were treated with a solution of soluble boron (boric acid 5g/L). This was applied as a foliar spray at each of the field sites to make a preliminary assessment of the effect of boron on petiole blackening.

Wasabi was grown at these field sites to maturity at 2 years of age. The crop was grown without the use of chemical pesticide treatments with the exception of Methiocarb (20g/kg) or Metaldehyde (15g/kg) snail and slug deterrents being applied as required.
Some leaves showed symptoms of white rust (*Albugo sp.*) during spring but removal of affected leaves prevented spread of this disease.

Aphis identified in a localised area at one of the field sites in September 2000 were controlled with a single application of 500g/kg pirimicarb.

In the winter of 2000 some premium quality stems were found to be damaged by rats or mice. Bait stations were set to prevent further damage prior to harvest.

Plant samples showing blackened lesions were collected in November 1998, February 1999 and September 2000 and sent to either Crop Health Services, Institute for Horticultural Development, Knoxfield, Victoria or DPIWE Newtown Laboratories, for pathological analysis.

### 3.3 Market evaluation of stems

In July and September 2000, samples of wasabi from the two semi-commercial production sites were test marketed prior to commercial sale. Eleven restaurateurs or food processors were chosen to evaluate the product.

A sample of four wasabi stems, clearly numbered (1 to 4) were chill-packed and delivered by express courier to each of the various market contacts in Hobart, Launceston, Melbourne and Sydney a day after stems were harvested. Each stem in the samples was measured, weighed and recorded prior to packing.

Telephone surveys were conducted by the Development and Marketing Officer of DPIWE Vegetable Branch over consecutive days. The survey was conducted with the end user or key decision maker/purchaser of the wasabi. Each interview took from 45 minutes to an hour to complete.
4. Detailed Results

4.1 Location of plant material

Harvest of wasabi crops from the seed imported from Japan began in July 2000 with a yield of 10 tonnes per hectare being recorded. The yield of premium quality stems was lower than expected (4 tonnes/ha) with processing grade material making up the remainder. One factor contributing to the low yield of premium stems was the variation in plant morphology observed at maturity.

Approximately 25% of plants produced from 1 to 5 premium quality stems, the remaining 75% of plants produced multiple stems that were too small for the fresh market but were suitable for processing as wasabi paste. This problem was attributed to the genetic variation within the imported seed.

4.2 Assessment of agronomic techniques

Propagation from seed

Wasabi seed has a soft seed coat so must be kept moist at all times making seed harvesting, cleaning and storage difficult. Storage at low temperatures reduces fungal contamination whilst satisfying the chilling requirement for germination causing stored seed to germinate even at 1°C. Results of seed storage and germination experiments are described below.

Experiment 1. To test the viability of wasabi seed following desiccation.

One hundred grams of fresh seed pods yielded 10.5 grams of dried seed with 400 seed per gram.

This experiment confirmed that wasabi has unique requirements for storage and handling. No germination occurred after seeds were dried to a moisture content of 20%. The seed retains viability when stored at a humidity level of 80 to 90%.

Experiment 2. To test viability of seed produced along the length of the raceme.

Traditionally wasabi is self-seeding with mature pods dehiscing after lying on damp soil or in running water for several weeks.

In Tasmanian trials wasabi demonstrated two flowering times annually, in spring and autumn. In order to harvest the maximum amount of seed, racemes were collected 10 weeks after flowering when basal pods on the raceme began to dehisce. The pods at the base of the raceme open first and the remainder open sequentially along the length of the raceme as the pods mature (See Figure 4).
Figure 4. Seed pods mature along the length of the raceme

Pods were divided into 5 groups based on their apparent maturity which decreased from the base of the raceme, nearest the stem, to the growing point at the top. Pods at the base of the raceme (section 1) had begun to dehisce, the remainder had not. Pods were progressively smaller for each of the 4 remaining sections. Because whole pods were used in this experiment, the number of seeds was not counted. The results are expressed as percentage of seeds germinated relative to the estimated number of seeds for each of the 5 sections. Wasabi seed pods average 8 seeds per pod. Results of this experiment are recorded in Table 1.

Table 1. Effect of pod maturity on seed germination.

<table>
<thead>
<tr>
<th>Raceme section</th>
<th>% Germination at 4°C</th>
<th>% Germination at 15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Base (pods dehiscing)</td>
<td>20</td>
<td>10-20</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10-20</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5  Top</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Although there was little difference in the germination percentages at each temperature, this experiment demonstrated that the seed from the base of the raceme produced more viable seed after 6 weeks storage at 1°C than did pods from the remainder of the raceme. However, additional seed attached to the raceme was harvested at the same time and stored for up to 19 weeks at 1°C. Two cell trays (96 seeds) were planted at 3 weekly intervals from 7 to 19 weeks and showed increasingly higher germination percentages (up to 90%) with time in storage. Seed matured sequentially along the length of the raceme even at 1°C.

Experiment 3. To establish a commercially viable method of seed extraction (see Figure 3)

In order to produce pod free seeds ready to sow the treatment times for the four methods varied as described:
1. Seed pods stored in running water at 12°C for 4 weeks.
2. Seed pods stored in aerated water at 12°C for 6 weeks.
3. Seed pods placed in a sieve and continually raised and lowered in aerated water at 12°C for 10 days.
4. Seed pods placed in water-filled cylinder into which air was introduced under pressure for 5 days.

Method 4 was the most efficient for extracting seeds from the pods. This method was rapid, provided gentle agitation without desiccation and yielded clean seed ready for sowing. The longer treatment times for methods 1 and 2 resulted in some seed loss due to the material rotting.

Experiment 4. To test the effect of ventilation and removal of pods from raceme on seed viability.

In November 1999 seed harvested from the field sites was stored at 1°C for 7 weeks then cleaned using method 4 described above in Experiment 3, and sent to a commercial nursery for propagation. However the nurseryman reported germination percentages of less than 1%. An explanation for the low germination percentages was sought.

The seed used in Experiment 2, was harvested in November 1998 and stored with pods still attached to the raceme in open plastic bags containing moist paper towel. However, the seed used in the seed cleaning experiment (Experiment 3) harvested a year later, had been removed from the racemes when harvested and stored in sealed plastic bags. The influence of the raceme and ventilation of seed during storage was investigated.

This test aimed to determine the influence of the raceme (flower stalk) and ventilation on seed germination. Forty-eight seeds from each treatment were sown. The results of this experiment are shown in Table 2.

Table 2. Effect of ventilation and raceme on seed viability

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of seeds germinated</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stalked, unsealed</td>
<td>35</td>
<td>73</td>
</tr>
<tr>
<td>Stalked, sealed</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Destalked, unsealed</td>
<td>25</td>
<td>52</td>
</tr>
<tr>
<td>Destalked, sealed</td>
<td>15</td>
<td>31</td>
</tr>
</tbody>
</table>

This experiment demonstrated that storage of seed pods in sealed plastic bags resulted in lower germination percentages than storage in open bags. The provision of an adequate supply of oxygen for stored seed was implicated. In addition, the seed pods which remained attached to the raceme had higher germination percentages than those removed from the raceme. This indicates that the raceme assists in maturation of seed during storage as indicated in Experiment 2.

Experiment 5. To test the influence of gibberellic acid on seed germination.

Wasabi seed exhibits strong dormancy and will not germinate when freshly harvested (Chadwick et al. 1990). Dormancy can be artificially broken by gibberellic acid, cold treatment or stratification (Chadwick et al. 1990; Palmer 1990).

Seed harvested in November 2000 was treated with 400 ppm gibberellic acid (GA) for different periods ranging from several hours to several days. Fifty seeds were used for each treatment. All seeds were from the same plant. The number of germinating seed was counted after 7 days. The results are recorded in Table 3.
Table 3. Effect of gibberellic acid on seed germination

<table>
<thead>
<tr>
<th>Time of exposure to GA (hours)</th>
<th>Number of germinating seed</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>24</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>48</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>72</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>120</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

These results indicate that gibberellic acid may be useful in breaking seed dormancy in wasabi and might be used to reduce seed storage time prior to germinating seedlings for subsequent crops.

Seedless propagation

Micropropagation

The commercial tissue culture laboratory 'Agronico' was engaged under contract to investigate the development of wasabi plantlets using micropropagation techniques. At the conclusion of the contract, wasabi had been successfully initiated in culture but the material had not begun to proliferate. A second contract was established with 'Brio' commercial laboratory, with the same laboratory technician involved. Problems with fungal and bacterial contamination hindered progress with shoot proliferation. Expertise was sought from the micropropagation laboratory of Tasmanian Institute of Agricultural Research. Recent reports from the TIAR laboratory indicate clean tissue has been maintained in culture, shoot proliferation has been achieved and plantlets subdivided. Reports from the 3 tissue culture laboratories involved with the wasabi project appear in Appendix 1.

Vegetative Propagation:

Wasabi produces side shoots which can be easily removed from the main stem. These can be readily propagated by placing in moist sand or well-aerated water. Literature from Japan (Chadwick, 1990 after Suzuki et al. 1976) recommends that side shoots be taken from subsequent populations for a maximum of two years to prevent the build up of disease in the crop. Plant losses in the field were replaced with splits from established plants. These regenerated readily and reached maturity in a shorter time than did plants grown from seed.

After the first winter, when the plants were approximately 15 months old, the rhizome or stem of the wasabi plant began to elongate. This allowed identification in the field of plants with superior morphological characteristics. These plants had 4 or 5 crowns which had the potential to develop a stem suitable for the fresh market. Twenty-six plants were selected and isolated to a site on a nearby property for cross-pollination purposes. Vegetative side shoots were also taken as splits from the selected plants and grown in a shaded glasshouse at Mount Pleasant Laboratories. After 12 months the selected plants produced seed which was harvested from both field and glasshouse plants and stored at 1°C. Sowing of this selected seed commenced in January 2000 and will produce seedlings for the next Tasmanian wasabi crop.
Semi-commercial production

The first field site was a level site with a deep alluvial loam soil which was flood irrigated. Previous use of this site as a hop field lead to a reduction in establishment costs as the shade house was erected used existing wires and posts. Early plant losses were high at this site due to poor soil drainage causing waterlogging. A change to overhead sprinkler irrigation allowed more even distribution of water over the site and was immediately effective in reducing plant loss.

The second site was a gently sloping krasnozem soil overlaying deep granitic loam. Overhead microsprinklers supplied irrigation water at this site and plant losses were minimal (Figure 5).

The fertiliser trials demonstrated that wasabi plants respond favourably to NPKS fertiliser with plants responding best to PIVOT 800® formulation NPKS 8:11:10:7 applied at a rate of 1260 kg/ha. Plants grown with fertiliser at the lowest application rate did not thrive as did plants at the second and highest rates, so the fertiliser trial was terminated and the two sites top-dressed with PIVOT 200® at 400 kg/ha. This ensured that sufficient quantities of the major nutrients were available throughout the 2 year growth cycle of the crop. Nitram® (33% N) fertiliser applied at 3 monthly intervals a rate of 100 kg/ha promoted crop growth especially during the rapid winter growth phase.

Blackened lesions which became apparent on the petioles of wasabi during the summer resembled the symptoms described for boron deficiency in brassica vegetables (Shorrocks [1982]; Weir and Cresswell, 1993). The results of a single application of foliar boron on wasabi at 12 months are detailed in Table 4.

Table 4. Effect of foliar boron on petiole lesions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plants with blackening</th>
<th>Total plants</th>
<th>Blackening %</th>
</tr>
</thead>
<tbody>
<tr>
<td>No boron</td>
<td>45</td>
<td>77</td>
<td>58</td>
</tr>
<tr>
<td>Boron</td>
<td>33</td>
<td>70</td>
<td>47</td>
</tr>
</tbody>
</table>

Although the results of this preliminary investigation were not conclusive, they do indicate that boron deficiency may be contributing to plant stress causing petioles to become darkened, brittle and cracked, opening the interior tissue to infection. Further investigations into the effect of regular applications of foliar boron may be beneficial.

Leaf samples showing some darkened spots were collected in November 1998 and sent for pathological analysis by Crop Health Services Institute for Horticultural Development, Knoxfield, Victoria. The conclusion was that the lesions were being caused by the fungus *Phoma lingum*. The brown leaf spots became less prevalent as the crop matured and no treatment was applied.

In February 1999 a sample of plants with general wilt symptoms were collected and analysed for pathological disorders by DPIWE Newtown Laboratories. This analysis reported no evidence of *Phoma sp.* in the samples but the fungus *Fusarium* was reported to be causing secondary infection leading to wilt symptoms. Bacterial parasites had also invaded the infection site. As the incidence of plants with wilt symptoms was low (about 5%) no pesticide control measures were applied.

In September 2000 a sample of plants showing blackened lesions on the petioles and stems were sent to Crop Health Services Institute for Horticultural Development, Knoxfield Victoria. This laboratory again reported the incidence of *Phoma lingum* in the samples.
A 'Wasabi Production Guide' (Appendix 2) has been prepared for interested growers which gives details on site establishment, nutrition and irrigation requirements, recommendations for controlling pests and diseases and procedures for harvesting and preparation of stems and leaves ready for market.

![Wasabi crop 21 months after sowing](image)

### 4.3 Market assessment and evaluation

A number of leads for potential markets for wasabi have been identified. In addition to looking at the Japanese arena with a number of industry contacts, market discussions with Australian food service and value-adding organisations have been conducted.

**Market survey**

Samples of fresh wasabi stems from the two semi-commercial production sites were test marketed in July 2000 prior to commercial sale (Figure 6). Eleven restaurateurs or food processors were chosen to evaluate the product and their responses were extremely favourable. A summary of the survey (Appendix 3) and a copy of the market survey questionnaire are included in this report (Appendix 4).

The Tasmanian product is considered to be greatly superior to frozen wasabi stems imported from Japan and also to the prepared pastes and powders currently available. The survey also indicated that although fresh wasabi attracts a premium price, processed wasabi products such as prepared paste and wasabi powder are also sought by the catering industry.

One of the characteristics of Tasmanian wasabi which the survey indicated might be improved was that the flavour should 'linger on the palate'. A review of the available literature was made to identify the flavour components of wasabi.

The databases CAB and CAPLUS provided useful information regarding the flavour compounds of wasabi. The Central Science Laboratory at the University of Tasmania completed chromatographic analyses of Tasmanian wasabi stems with favourable comparisons of most flavour components described for wasabi grown in both Japan and New Zealand. (Depree et al. 1999). The group of components that are deficient in Tasmanian wasabi may be due to a varietal characteristic or it may be attributed to differences in crop husbandry. Appendix 5 describes the analysis of volatile components of Tasmanian wasabi.
One commercial distributor, Greengrocer.com, marketed fresh Tasmanian wasabi from the trial sites for the six months from August 2000 to January 2001 at a price of $100/kg. In addition to the wasabi stem, there is demand from the market place for wasabi leaves and petioles. These were also marketed from the trial plot at $5/bunch of 10 leaves, directly to a Japanese restaurant in Sydney.

Samples of processing grade stems have been sent to a number of food processing companies for evaluation as a prepared paste. Samples of dried 100% wasabi powder are being tested for inclusion in a dried spice range. Tasmanian wasabi, both fresh and powdered, was also successfully trialled by a small Tasmanian cheese manufacturing company for inclusion in a new product line.

These value-adding organisations are keen to trial large quantities of Tasmanian wasabi in new products. They require quantities of approximately 5 tonnes which will be beyond Tasmania's production capacity for at least 4 years.

Continued market assessment provides valuable information regarding product volume, consistency and quality. A business plan for the production and marketing of Tasmanian wasabi was 1 of 10 finalists in the RIRDC Business Plan Competition in November 2000. A commercial in confidence copy of this plan is available to RIRDC.
**Promotion**

Although the Tasmanian wasabi industry is in its infancy, there has been overwhelming interest from both the marketing sector and potential growers. Table 5 lists the fora at which wasabi has been presented. Publications made available at these fora are presented in Appendix 6.

**Table 5. Fora for presentation of Tasmanian wasabi**

<table>
<thead>
<tr>
<th>Forum</th>
<th>Venue</th>
<th>Date</th>
<th>Patrons</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPIWE Vegetable Branch field day</td>
<td>Forthside Vegetable Research Station</td>
<td>February 9, 2000</td>
<td>100</td>
<td>Commercial growers identify wasabi</td>
</tr>
<tr>
<td>'Taste the Harvest' Food &amp; Wine festival</td>
<td>Devonport Tasmania</td>
<td>April 16, 2000</td>
<td>5000-7500</td>
<td>Public familiarisation</td>
</tr>
<tr>
<td>'Tasmanian wasabi' Powerpoint presentation followed by tasting</td>
<td>DPIWE Prospect Offices, Launceston</td>
<td>April 13, 2000</td>
<td>Japanese Ambassador Mr Masaji Takahashi, Mrs Takahashi and entourage</td>
<td>Introduction of Tasmanian wasabi served at Japanese Embassy</td>
</tr>
<tr>
<td>'Agfest' Tasmanian agricultural show</td>
<td>Carrick, Tasmania</td>
<td>May 4-6, 2000</td>
<td>50,000</td>
<td>Public and growers identify wasabi</td>
</tr>
<tr>
<td>National Restaurant and Caterer's Convention and Trade Exhibition</td>
<td>Wrest Point Casino, Hobart</td>
<td>October 9, 2000</td>
<td>250</td>
<td>Fine food market aware of local product</td>
</tr>
<tr>
<td>Alternatives in Agriculture Forum</td>
<td>Launceston, Tasmania</td>
<td>November 14, 2000</td>
<td>50</td>
<td>Familiarisation for growers and scientists</td>
</tr>
</tbody>
</table>

**Media articles**

Following the sale of small quantities of fresh stems from the first semi-commercial harvest there has been significant media exposure for Tasmanian wasabi:

- Interviews on ABC National radio programs 'The Country Hour' and 'Drive Time'.
- 'Panorama' the in flight magazine of Ansett Australia and Air New Zealand, February 2000 issue.
- 'Agora' the in flight magazine for Business Class travelllers with Japan Airlines Company Limited, Autumn 2001 issue.
- 'Australian Gourmet Traveller' May-September 2001 issue (in Press).

Full reference details for these articles are presented in Appendix 7.
5. Discussion of Results

5.1 Plant material

Harvest of wasabi crops began in July 2000 with yields of 10 tonnes /ha being recorded. The yield of premium quality stems was lower than expected with two problems being identified:

1. **Variation in plant morphology:** Approximately 25% of plants produced from 1 to 5 premium quality stems, the remaining 75% of plants produced multiple stems that were too small for the fresh market but were suitable for processing as wasabi paste.

2. **Blackened stem lesions:** These reduced the marketability of approximately 50% of premium stems to processing status. The cause of the blackened lesions has not been fully identified although plant stress and pathological disorders have both been considered following analysis of affected plants.

In Victoria, Australia, the Murrindindi Shire Council has commenced development of a wasabi crop with the financial support of ITO EN, a Japanese supermarket outlet. Discussions with the Director of Planning and Economic Initiatives at the Murrindindi Shire Council in Victoria resulted in one of their delegates visiting Tasmania in November 1998. Subsequently, a semi-formal offer was made to the DPIWE to work closely on the existing project with ITO EN especially in the area of plant propagation.

A visit was made to the Victorian site, by the Tasmanian grower, Principal Investigator and Project supervisor in November 2000. The Victorian trial, conducted in an artificial streambed, is approximately 200 square metres in area and uses *Wasabia japonica* variety 'Midori'. Plant maturity was 12 months behind that of the Tasmanian crop and similar problems with blackened lesions and seasonal variation in seed germination were evident. At 12 months of age variety 'Midori' exhibited more desirable morphological features than the Tasmanian variety 'Daruma'. Plans to exchange planting material have already been discussed. An assessment of the percentage of premium quality stems produced by this variety at maturity would be valuable. Victorian and Tasmanian growers agree that cooperation in development and marketing of wasabi would be beneficial for both the Australian and export industries.

5.2 Agronomic assessment

Wasabi seed exhibits strong dormancy and will not germinate when freshly harvested (Adachi 1987; Tatsuyama et al 1983). In conducting these experiments it was noted that seed produced from the spring flowering had lower germination percentages than that produced from the autumn flowering. The most likely explanation for this is that seed produced from the autumn flowering matures in the field when temperatures are low thus partially satisfying the chilling requirement (Palmer 1990) before the seed are harvested. Seed produced from the spring flowering matures when temperatures are rising and so require a longer chilling treatment post harvest. According to Chadwick et al. (1990) the depth of dormancy is a function of air temperature during maturation on the mother plant.

The most likely explanation for the poor germination percentages reported by the commercial nursery are a combination of factors:

1. Seed pods were removed from the raceme inhibiting further maturation of the seed.
2. Seed pods were stored in sealed plastic bags which provided inadequate ventilation.
3. In cleaning seed, the seeds were removed from the pod possibly further reducing the seed maturation process.
4. Seed was stored for only 5 weeks at 1°C, whereas longer term storage was found to promote germination.

5. Seed was produced from spring flowering.

Investigations into propagation of wasabi using vegetative or tissue culture techniques were precipitated by the difficulties involved in handling and storing the seed. Vegetative propagation has the potential to produce a crop which has the genetic characteristics of selected plants. Plants chosen for vegetative propagation were those with a growth habit which produced several large stems at maturity. Seed from these selected lines were collected from both the field and glasshouse plants in November 2000 and will be used to establish a subsequent Tasmanian wasabi crop.

In addition to seed production, side shoots from plants with desirable stem characteristics were retained during the harvest of semi-commercial crops in July 2000 and planted in a sand bed for propagation purposes. Both seed and vegetatively produced plants will provide a source of selected material for micropropagation. The establishment of protocols for micropropagation for wasabi will facilitate commercial production of planting material of premium quality.

Blackened lesions on petioles were observed during the summer months when growth was slow but were not obvious during the winter when the plants made the most rapid growth. Samples sent for pathological analysis were reported to be exhibiting symptoms of secondary infection. Poor drainage together with high summer temperatures has also been suggested as a cause of the blackening on petioles, which become cracked causing the plant to become susceptible to fungal and bacterial infection. The incidence of stem blackening is greater where plants are subject to poor drainage.

The blackened lesions on petioles of the wasabi plant resemble those caused by boron deficiency in brassica vegetables. Since wasabi belongs to the same plant family as brassica vegetables and has a much longer growth cycle it is possible that boron becomes deficient before the crop comes to maturity and the blackening extends into the developing stem. Whilst the preliminary experiment described in this report was not conclusive, further field trials may show that regular applications of foliar boron solution during crop growth may be effective in reducing the incidence of stem blackening.

As wasabi is a new crop to Australia, no pesticides have previously been registered for use on this species. Application was made to the National Registration Authority to classify wasabi into the group ‘brassica vegetables’ based on the criteria described in the Codex Alimentarius Commission’s ‘Guide to Codex Classification of Foods and Animal Feeds’. This application was successful and allows wasabi to be treated with agricultural chemicals which are currently registered for use on brassica vegetables. Permits for off-label use of other agricultural chemicals which may be required to treat potential pests have also been sought and approved. Data from efficacy trials will be provided for the National Registration Authority if these chemicals are used. For the two year growth cycle of the crop, wasabi was treated only to control snails and slugs with growers keen to maintain a low incidence of pesticide treatment in producing this crop.
5.3 Market evaluation

The harvest and marketing of material from the first semi-commercial trial crop was met with great enthusiasm by the domestic market. The Australian restaurant trade has previously had access only to frozen wasabi imported from Japan or prepared wasabi paste much of which is combined with English horseradish. The results of the market survey were very encouraging and helpful in recognising components of the Tasmanian product which could be improved upon. These include the hot taste lingering on the palate, the stem being greener in colour and evenly tapered.

Some of the characteristics may become more dominant in the existing variety if the plants are grown aquatically. This production method is more costly but produces the best quality stems or rhizomes (Depree et al 1999). Water-grown production lends itself to greater control over the root temperature of the plant, keeping the temperature in the optimum range of 12-15°C. Slow uniform growth rate would allow development of evenly tapered stems, cooler temperatures are also beneficial to the production of a full complement of flavour compounds. Reflected light from the surface of the water would allow the stem to become more photosynthetically active and therefore greener.

Alternatively, new varieties of wasabi may need to be introduced to obtain the required improvements in taste characteristics. These varieties may be introduced from Japan, New Zealand or Victoria. Discussions have already started regarding the introduction of stem material which might be used for vegetative or micropropagation purposes. Chromatographic analysis of the flavour components of introduced stem material would be valuable in determining varietal influences on flavour components. A comparison of the performance of the new varieties with the Tasmanian variety ‘Daruma’ grown in soil or water production systems would be valuable in varietal selection for the future.

During the harvest of mature wasabi stems in July 2000 the incidence of blackened stem lesions was noted. These reduced the marketability of approximately 50% of premium stems to processing status. The cause of the blackened lesions may be directly attributable to fungal infection (eg. Phoma) or it may be caused by plant stress which makes the plant more susceptible to fungal infection (eg. Fusarium).

The symptoms of leaf spots, petiole blackening and wilt all occurred during the summer months which may indicate that warm temperatures contributed to plant stress. These symptoms were not observed during the cooler months from March to October when the wasabi crop grew most rapidly.
6. Implications

Wasabi production occurs in specific areas where environmental constraints of air temperature and water quality are satisfied. Areas suitable for soil and water grown wasabi have been identified in the north of Tasmania. To date Tasmanian wasabi production has been based on soil culture in order to establish fertiliser and crop protection requisites for this crop which is new to Australian cultural systems. There has been considerable expansion in interest from both consumers and growers in the development of this niche industry.

Marketing of Tasmanian wasabi from the semi-commercial field trials has met with an overwhelming response. Clearly the domestic market is keen to purchase high quality fresh wasabi and the Tasmanian product is well accepted by a number of first class restaurateurs.

Value-adding organisations are also keen to have access to large volumes of wasabi for inclusion in a range of products previously not available on the domestic or international markets.

Black lesions are currently reducing the marketability of up to 50% of stems for the fresh market although much of this material is still suitable for processing as wasabi paste. Reducing the incidence of blackening would significantly improve the financial viability of the crop as would identification and propagation of stems with superior physical and taste characteristics. A 4-fold increase in premium stem yield may be achievable when stem blackening is eliminated and plants from selected lines are used as planting material (See Table 6).

Table 6. Effect of black stem reduction and superior plant selection on profitability

<table>
<thead>
<tr>
<th></th>
<th>Premium Stems</th>
<th>Processing Stems</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current Results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Plants (/ ha)</td>
<td>9,000</td>
<td>17,000</td>
<td>9,000</td>
<td>35,000</td>
</tr>
<tr>
<td>Stem Yield (t/ha)</td>
<td>4</td>
<td>6</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Gross Return ($/ha)</td>
<td>400,000</td>
<td>120,000</td>
<td>—</td>
<td>520,000</td>
</tr>
<tr>
<td><strong>Targeted Results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Plants (/ ha)</td>
<td>35,000</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stem Yield (t/ha)*</td>
<td>16</td>
<td>4</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>Gross Return ($/ha)</td>
<td>1.6m</td>
<td>80,000</td>
<td>—</td>
<td>1.68m</td>
</tr>
</tbody>
</table>

*Processing stems will be produced as a consequence of grading.

Development of protocols for micropropagation will permit the rapid establishment of the subsequent wasabi crop by making available plants with superior characteristics. It will also initiate the development of a tissue culture business which can supply high quality virus free planting material to the expanding wasabi industry.
7. Recommendations

7.1 Visiting expert

*Recommendation: A relevant expert in wasabi production be invited to Tasmania*

It is recommended that an expert in wasabi production be invited to Tasmania from Japan or from Washington USA where production protocols suitable for western agricultural systems have been established. This would be beneficial to developing the local industry as there is limited literature available that addresses cultural practices. In particular advice on aquatic husbandry would be considered valuable.

A delegation from The Japanese External Trade Organisation (JETRO) visited the Tasmanian wasabi sites early in February 2000. This agency offered to assist in identifying a suitable expert in wasabi production who could visit Tasmania and offer cultural advice.

Contacts in Japan are also being sought through one Japanese restaurateur in Sydney who has purchased Tasmanian wasabi stems and leaves from the trial plots. Professor Rob Clark from the University of Tasmania's School of Agriculture has offered advice regarding contacts in Washington State USA where wasabi is produced using western style agricultural systems.

7.2 Improve plant quality

*Recommendation: A comparison of a range of genetic material be conducted to address market feedback on stem flavour and appearance.*

As a result of semi-commercial production of wasabi the Principal Investigator recommends that new varieties of wasabi should be introduced from Japan and Victoria, Australia to compare with variety 'Daruma'. Comparison of volatile flavour components in fresh premium quality stems can then be made by chemical analysis (chromatography) and selection of material for micropropagation based on both flavour components and stem morphology.

The importation of fresh wasabi stems from either Victoria or Japan has been discussed with quarantine officers of DPIWE and a procedure outlined for vegetative propagation from this material under quarantine conditions. The use of stem material for propagation purposes may be of greater benefit than the importation of more seed.

Establishment of protocols for micropropagation of wasabi should be completed and documented for use by a commercial tissue culture laboratory.

Continued liaison with the Victorian grower to compare both morphological features and market assessment of variety 'Daruma' with variety 'Midori' is recommended. In particular, a visit to the Victorian site when the crop is ready for harvest would be an advantage. Comparison can then be made regarding stem morphology, flavour and yield of premium versus processing grade stems.
7.3 Identify cause of stem blackening

Recommendation: Conditions which exacerbate stem blackening be identified by conducting glasshouse and field trials

The incidence of blackened lesions increases when the plants are under stress with a number of factors thought to contribute to these symptoms. These include boron deficiency, poor drainage and high soil temperature. Such factors may be directly responsible for the observed blackening, or they may make the plants more susceptible to fungal infection. Glasshouse trials should be conducted to confirm which factors contribute to blackened stem lesions ideally using genetically identical plant material propagated from vegetative side shoots or by micropropagation. Field trials to examine the effect of protective fungicide in reducing the incidence of blackening would also be valuable.

The effect of stem blackening on the yield of the Victorian crop would make a valuable comparison with the Tasmanian crop.

7.3 Aquatic wasabi

Recommendation: Production protocols for aquatic wasabi be established.

Aquatic wasabi is considered a higher quality product on the Japanese market than is soil-grown wasabi. Areas in Tasmania suitable for semi-commercial aquatic wasabi production have been identified, as have growers keen to establish production systems based on either diversion of natural streambeds or hydroponic systems.

During the course of this project wasabi has been grown by the DPIWE in a hydroponic system on a small scale with great success. The plant grows well with a nutrient complement similar to that of hydroponically produced tomatoes. One of the reasons for the success of this model lies in the provision of a hydroponic solution which is maintained at a temperature of 12-15°C, a condition of root temperature which the literature recommends is a necessary prerequisite for wasabi production (Palmer, 1990; Depree et al. 1999). Production of aquatic wasabi should be the next developmental step for the Tasmanian industry.
8. Appendices

8.1 Appendix 1: Micropropagation Reports

Appendix 1.1 Final report from 'Agronico' commercial laboratory December 1999

Proposal for Additional Work on *Wasabia japonica*

Whilst the establishment of *Wasabia japonica* into tissue culture did prove successful, the failure of the *in vitro* plantlets to multiply requires further investigation.

This is therefore a proposal outlining the possibilities of further investigation in order to establish a method of rapid multiplication for *Wasabia japonica*.

Whilst a sterilisation technique and initiation method of wasabi into tissue culture has been established, investigation into media components and growth hormones is now required in order for *in vitro* plantlets to multiply.

Media

Establishment of *in vitro* wasabi has proven successful in modified potato M&S media however, a move toward a more traditional form of M&S media may well prove more beneficial in promoting multiplication.

It is therefore proposed that a trial will once again be undertaken using traditional M&S media for wasabi tissue culture, whilst varying the plant growth hormone supplement.

Plant Growth Hormones

Previous research resulting from a literature search on *Wasabia japonica* in tissue culture suggested that rapid multiplication was achieved using only cytokinins, namely BAP.

Failure of this in previous trials now leads to the assumption that rapid multiplication of wasabi may well be achieved using both cytokinins and auxins.

Therefore it is proposed that a trial to investigate this should include varying levels of both BAP and the auxin NAA at levels ranging from 0.1 mg/L. A supplementary trial involving both IAA and IBA (at comparable levels) may also be investigated.

Finally it is proposed that this trial will be undertaken using both immature and older (up to 3 year) plant material, in order to determine if more established older plant material will create a more stable *in vitro* culture of *Wasabia japonica*.

*Wasabia japonica* Tissue Culture Literature Review

An initial literature search was conducted covering aspects of wasabi tissue culture. This search was carried out by the Tasmanian State Reference Library, recovering a total of eleven articles on the subject, seven of which were either in Korean or Japanese, the remaining four articles being in English. Of the original eleven, only three were specific to this project, covering the technique and media application for initiating *Wasabia japonica* into tissue culture for rapid multiplication.
The three relevant articles were in Korean and consequently required interpretation. This was attempted through the University of Tasmania however the results were rather vague.

Of the Korean articles, all of the abstracts were in English thus providing the only form of information in beginning a *Wasabi japonica* culture. Of these articles, Seon et al (1998) and Lee et al (1994) both utilised similar methods with growth hormones, BA and NAA at a rate of 0.2 - 1.0 mg/L being investigated. Eun et al (1998) also experimented with these growth hormones, however also utilised the cytokinin Kinetin at a rate of 0.2 – 1.0 mg/L, however this was not as successful.

All authors used MS medium, and used comparable sterilisation methods, however each initiated different sections of the plant. Seon et al (1998) initiated apical meristems of *Wasabi japonica*; Eun et al (1998) worked with flower stalk nodes and Lee et al (1994) initiated axillary buds, all reporting varied success.

Additional literature was also sourced from the internet, however information on wasabi tissue culture was merely mentioned with no details provided. Information was also gained through a tissue culture unit offered in a New Zealand high school. This information was given in the form of a unit curriculum outline for both students and teachers. Whilst the information was somewhat basic, follow up correspondence from the teacher involved has provided an avenue of further research and investigation.

References


Appendix 1.2  Interim report from Brio Laboratory


May 29:

Previously abandoned seed initiation trials were revisited. A more rigorous sterilisation technique was adopted due to the high contamination of previous trials. This involved the rinsing of seeds in a 70% ethanol solution prior to immersion into a 2% sodium hypochloride solution for an extended period of 12 minutes with continuous agitation.

Unfortunately this experiment whilst presenting a lower rate of contamination (38%) did again fail to initiate germination and those which did emerge from the seed coat failed to progress beyond that point.

The reason for this may be that the seed source was the batch used in previous trials (dated April 27) which were later classified as non viable seed stock.

June 19:

A combined liquid culture experiment was initiated. This employed liquid M&S media (that is containing no agar) of 20ml in 250ml containers supplemented with 0.5 mg/L 2,4-D. 2,4-dichlorophenoxyacetic acid is a purely synthetic compound which acts as an auxin with a similar physiological action of IAA. The reason for using 2,4-D as opposed to IAA is that this synthetic compound acts more profoundly and rapidly in the production of reproductive cell matter.

The aim of this experiment was to encourage the production of callus which may then be differentiated on solid media containing both auxin and cytokinins to produce shoots.

This experiment included both root tips, leaf sections, whole leaves and stems sections. They were sterilised in a 2% sodium hypochloride solution with sterilization times being varying according to the nature of the explant. The sections were then rinsed four times in sterile water. Cultures were incubated in 20°C with a 16 hour photoperiod and constantly agitated using an orbital shaker.

The experiment was abandoned 48 hours later when it was discovered that the cultures were in fact simmering due to the fact that the plate of the orbital shaker was heated and effectively cooking the liquid media and the explants.

June 27:

The initiation of wasabi seeds sourced from a new batch of seed grown by DPIWE was initiated. This experiment employed the same media as used for the liquid culture experiment however it was solidified with agar and used petri dishes.
A total of 150 seeds were divided into two groups prior to surface sterilisation. Each group was subjected to different sterilisation techniques. The first was insulated with foil and sterile cotton wool and sterilised in the autoclave at 120 °C for 18 minutes before initiating onto petri dishes. The second group was subjected to the usual 2% sodium hypochloride sterilisation technique for a period of 10 minutes prior to initiating onto petri dishes.

Both seed lots were incubated at 20°C in complete darkness. Results showed an expected loss of seeds subjected to the 2% sodium hypochloride solution due to contamination. Whilst there was no contamination displayed on those seeds sterilised in the autoclave, no germination was observed. The explanation for this failure to germinate is likely to be that the sterilisation technique was too harsh.

August 4:

Wasabi root tips were excised from semi-mature plant material. Plants were removed from their pots and roots were washed clean of all soil mix before tips of larger dominant roots were excised and cut into 1-2cm long sections and surface sterilised. Sterilisation was done via an initial immersion into 70% ethanol followed by continual agitation in a 2% sodium hypochloride solution and rinsing in sterile water four times.

Root tips were then initiated onto plates containing a solidified M&S medium supplemented with 0.5 mg/L 2,4-D.

Root tip cultures are currently incubated at 20°C in complete darkness. They have to date displayed little contamination with a current loss of 3%. Whilst some cultures are displaying a blackening on the root tip surfaces not in direct contact with the media, this does not appear to be affecting their overall health in that they are still displaying signs of expansion and swelling indicative of cell multiplication which may produce callus.

Future experimental options:

Further root tip initiation experiments seems at this stage to be a viable option for investigating the establishment of wasabi tissue culture. This would aim to expand on the current experiment including a revising of sterilisation techniques to further reduce the current rate of contamination.

An investigation of inflorescence material as a possible source of culture material may also be investigated. Information on such an experiment is scarce, nevertheless it shall be trialled in the next two weeks.
Appendix 1.3  Final report from Brio Laboratory March 2001

October 2000:

A trial using wasabi inflorescence material was initiated using M&S media incorporating 2.0mg/L BA and 0.1mg/L NAA. This trial also incorporated a sterilisation treatment slightly harsher than those previously used. Explants were subjected to a sterilisation treatment which involved plunging them into 70% ethanol followed by a continuous agitation in a 2% NaOCl solution finishing with rinsing 4 times in sterile water.

A parallel experiment using the same media recipe as well as the same sterilisation technique was run in conjunction with the inflorescence trial. The only differences with this experiment were the type of explant (meristematic material) and the fact that these explants were stored in distilled water for a period of 24 hours in the refrigerator.

Both of these experiments failed within 8 days due to bacterial contamination.

November 2000:

Wasabi inflorescence explants were initiated using a sterilisation process of plunging explants into 70% ethanol followed by continuous agitation in 1.5% NaOCl and rinsed four times in sterile water. Explants were then initiated onto a revised rhododendron medium (Anderson, W.C., 1984). The reason for this was due to the revisions in inorganic nutrients such as nitrogen, potassium and phosphorus all of which were reduced and iron, which was enhanced. These changes reduced the salinity of the inorganics, and hormone or plant growth regulators were also included: 1mg/L IAA and 5mg/L 2iP.

Again, this experiment failed due to the unsuccessful sterilisation process resulting in bacterial contamination of explants within a two week period following inoculation.

December 2000:

Wasabi explants were initiated onto M&S media supplemented with hormone levels of NAA and BA as previously stated. The only difference noted in this media was the absence of agar so making the media liquid. A sterilisation process of 20 minutes agitation in soapy water followed by 1 minute in 70% ethanol, 12 minutes in 1% NaOCl and finished by a 4-fold rinse in sterile water unfortunately failed once again to adequately clean the explants. This experiment was terminated after 3 weeks due to contamination.

January–March 2001:

No new experiments to initiate wasabi into tissue culture have been embarked upon in 2001 following confirmation that another laboratory was achieving results in this field.

Conclusion:

Whilst several experiments have been tested concerning both media requirements and sterilisation techniques for wasabi in tissue culture, it is concluded here that sterilisation of explants is the largest hurdle to initiating a culture. There appears to be an endemic bacterial and fungal infection within the wasabi plants from which these experiments were initiated. This infection may well stem from within the soil in which these plants are grown and perhaps a solution to this is to attempt initiation from hydroponically grown plants.

Appendix 1.4  Report from TIAR March 2001
I have established two groups of cultures.

**Group 1**

These cultures are proliferations originated from eight meristems taken at random from 2 unselected plants supplied by you. The cultures originating from each meristem have been kept separate and are designated as “lines”. 20 tubes of small proliferations of these cultures were transferred in mid Feb. Subsequently, four of the lines have developed symptoms of bacterial contamination. The contamination became obvious once the proliferations were subdivided, which indicates that the bacteria were probably of endogenous origin rather than surface contaminants. One of the surviving cultures has been tested for the presence of contaminants and found to be clean but I am proposing to do a more thorough check of all remaining lines at the time of the next subculture.

**Group 2**

10 meristems originating from either W6 or W7 plants from isolations carried out in late January.

These cultures are yet to commence proliferating but on initial culture media appear free from contamination. Given the recent experience it is likely that endogenous bacteria may arise in some cultures. This is most likely to occur once the proliferations are subdivided. The meristems originating from the W6 plant consist mainly of callus at present and may not develop satisfactory proliferations.

**Protocols in use:**

*Media:*
Meristem isolation medium  
MS medium + 30 g/l sucrose + 80 mg/L adenine sulphate, 2 g/L phytagel  
ph 5.8. Shoot meristems less than 1mm in diameter were excised and transferred to above media.

Proliferation medium  
MS medium + 30 g/l sucrose + 80 mg/L adenine sulphate + 1 mg/l benzyladenine, 2 g/L phytagel, pH 5.8.
8.2 Appendix 2  Wasabi Production Guide

The 'Wasabi Production Guide' is a stand alone document.
Wasabi Production Guide

Prepared by Department of Primary Industries, Water and Environment

A Sparrow, M Buntain and M Barber
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1. Introduction to the plant

Wasabi (Wasabia japonica Matsumara) is a perennial herb belonging to the same family of plants as broccoli, cabbage and mustards, the brassica family. It grows naturally alongside mountain streams in Japan. Superficially, the vegetative parts of the plant resemble an oversized violet. It is evergreen and forms large clumps if left undisturbed.

Wasabi has large hairless, heart-shaped leaves on long leaf stalks or petioles. Aged leaves naturally die and fall away leaving knobby scars on the thick greenish coloured stem or rhizome. The plant produces side shoots from the base and stem. The enlarged stem is the most valuable part harvested for culinary use. The leaves and flowers can also be used in fresh salads.

Wasabi sends out long flower stalks in spring and autumn. The flower stalk (or inflorescence) is leafy and small white flowers appear at the tip. The inflorescence continues to elongate producing more flowers along its length. The flowers are cross-pollinated and following pollination the inflorescence topples over below the leaf canopy and elongates to a length of about 1.5 metres. Up to 8 seeds develop in the long pods or ‘siliques’ which develop from each flower.

2. Site selection

2.1 Soil type

Wasabi grows best on soils with an open friable structure. Excellent drainage is essential. Preferred soil types are:

(i) red soils such as Krasnozems or similar basalt derived red soils
(ii) sandy soils high in organic matter.

Soil pH should be close to neutral with pH in the range 6.5 to 7.0. This will reduce the likelihood of club root disease which is more common in brassica crops grown in acid soils.

2.2 Site and location

Wasabi can tolerate air temperatures ranging from mild frosts (-3°C) to 30°C. However the ideal root temperature range of 12-15°C may be a constraint in choosing a site. At temperatures below 12°C growth is slow and above 15°C the plant exhibits signs of stress making it more susceptible to disease.

A sheltered location will prevent physical damage to the plants and make the required shade structure less vulnerable. Access to plentiful irrigation water is essential.
Wasabi plant parts: The rhizome is more often called the stem
3. Planting and plant arrangement

Autumn and spring are the best times to plant young wasabi as temperatures are moderate and stress to young plants is reduced.

3.1 Plant density

Plants are arranged in raised beds 1.1 metres wide with three rows of plants per bed. The current recommended plant density is 9 plants/m$^2$ with 30 cm between plants in a row and 40 cm between rows.

3.2 Planting arrangement

The best arrangement is to have rows running down the slope to assist drainage.
Flowering wasabi with young developing pods or ‘siliques’ on long flower stem

A permanent shade structure for soil-grown wasabi
4. Shade and shade structures

4.1 Shading

In traditional culture wasabi grows naturally under the shade of deciduous trees. Leaves become bleached and leaf burn may occur if exposed to too much radiation. In Shizuoka, Japan, shading of commercial wasabi varies from none to as high as 80% shade.

Currently, the standard shade cloth used for wasabi is 80% black. The shade structure design should allow good air flow for cooling. It is recommended that wire mesh be used for the lower section of the walls to help provide additional ventilation and cooling whilst providing protection from animals and reducing wind damage to the shade house structure.

Ideally the shade structure for soil grown wasabi should be simple for easy relocation to allow for crop rotation and to prevent the build up of soil-borne diseases.

4.2 Contacts for shade cloth

<table>
<thead>
<tr>
<th>Company</th>
<th>Contact</th>
<th>Phone</th>
<th>Black @ 80% x 50m</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARLON</td>
<td>P.R.M. (Peter Maynard)</td>
<td>6343</td>
<td>$259.39 (Code 601 809)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(03) 0477</td>
<td>$480.74 (Code 600 802)</td>
</tr>
<tr>
<td>Gale Australia</td>
<td>ASCO agencies</td>
<td>6344</td>
<td>$328.50 (+10%GST)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(03) 7060</td>
<td>$657 (+10%GST)</td>
</tr>
</tbody>
</table>

*Prices as at 27.2.01
5. Nutrition and fertilisers

In Japan, soil grown wasabi is fertilised with compost such as rape seed cake and liquid manures. No nitrogen is applied to the young seedlings when first planted in the soil.

The appropriate soil pH is necessary to prevent nutrient deficiencies/toxicities and to help prevent club root disease. As a general rule of thumb to achieve a pH of 6.5 to 7.0, apply 1 tonne of lime/ha for each 0.1 unit of pH needed. e.g. if pH is 5.5 then 10 tonne/ha lime is required. This should be applied pre-planting and incorporated into the soil.

High quality wasabi should grow at an even rate. This means that a number of small applications of nitrogen over the growing season should be applied. The amount applied will depend on the history of the site and its organic nitrogen levels. As a guide, for an average site that has been moderately cropped, apply 100 kg/ha nitrogen. This can be applied as 3 applications of Nitram (33%N) at around 100 kg/ha (10 to 12 g/m²) for each application (September, November, February). Regular monitoring of plant vigour and colour plus plant tissue analysis will allow a more accurate program to be determined.

Phosphate (P) fertiliser should be incorporated into the soil prior to planting. Potash (K) fertiliser can also be applied at this time. Banding the fertiliser along the rows is desirable. A soil test result will indicate the amounts needed. As a guide, a soil with P levels over 150 ppm and K levels over 350 ppm will require annual maintenance dressing only - 25 kg P/ha or 300 kg/ha single super phosphate (8% P) plus 35 kg K/ha or 70 kg/ha muriate of potash (KCl - 50% K). In year 2, plants should be top-dressed in early spring.

<table>
<thead>
<tr>
<th>Fertiliser</th>
<th>Pre planting (kg/ha)</th>
<th>Post planting (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broadcast</td>
<td>Band Placed</td>
</tr>
<tr>
<td>Borax</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Boric acid</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Solubor®</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

For foliar application, dissolve the amount in 300 to 500 litres of water and add surfactant. Apply fortnightly over 6 weeks. Molybdenum can be supplied as sodium molybdate (Na2MoO4.2H2O) at 1 kg/ha (0.4 kg/ha Mo) or as a foliar application of sodium molybdate at 5.0 l/ha.

6. Trace element requirements

Brassica crops are particularly sensitive to boron and molybdenum deficiency. In the first season before planting, apply boron fertiliser using the following table as a guide. Each box gives an alternative method and rate.

For foliar application, dissolve the amount in 300 to 500 litres of water and add surfactant. Apply fortnightly over 6 weeks. Molybdenum can be supplied as sodium molybdate (Na2MoO4.2H2O) at 1 kg/ha (0.4 kg/ha Mo) or as a foliar application of sodium molybdate at 5.0 l/ha.
## 7. Deficiency symptoms in brassicas

The following table gives an indication of deficiency symptoms in vegetable brassicas. These may give an indication of likely responses in wasabi.

<table>
<thead>
<tr>
<th>Element</th>
<th>Leaf age affected</th>
<th>Symptom</th>
<th>Occurrence</th>
<th>Critical levels % or ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Deficient</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Old first</td>
<td>Pale green then yellow as age</td>
<td>Common</td>
<td>1.9</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Old leaves</td>
<td>Purple flush, drastic reduction in growth</td>
<td>Common</td>
<td>0.35-0.7</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Young</td>
<td>Interverinal chlorosis, cupping up or down</td>
<td>Common</td>
<td>0.11</td>
</tr>
<tr>
<td>Potassium</td>
<td>Old</td>
<td>Scorch and curl at margins and inteverinal</td>
<td>Common</td>
<td>0.25</td>
</tr>
<tr>
<td>Calcium</td>
<td>Young</td>
<td>Cupping, distortion, tipburn, ‘beaking’, leaf crinkle</td>
<td>Common</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal browning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>Old</td>
<td>Interverinal chlorosis, diffuse at first then may have red/orange tints</td>
<td></td>
<td>0.03-0.06</td>
</tr>
<tr>
<td>Iron</td>
<td>Young</td>
<td>General yellow then white, some green may remain along midrib</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>All</td>
<td>Interverinal chlorosis, speckled olive green</td>
<td></td>
<td>1.3</td>
</tr>
<tr>
<td>Zinc</td>
<td>Expanding</td>
<td>Pitcher like cupping with outcurved margins</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>Expanding and mature</td>
<td>Diffuse interverinal chlorosis</td>
<td>Very rare</td>
<td></td>
</tr>
<tr>
<td>Boron</td>
<td>New</td>
<td>Convex cupping , moribund growing point</td>
<td>Common</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>Interverinal chlorosis; cracked corky stems, brown heart</td>
<td></td>
<td>30-60</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Young</td>
<td>Interverinal chlorosis, bleaching near margin, leaves cupped and elongated, growing point becomes blind</td>
<td>Common</td>
<td>0.13</td>
</tr>
</tbody>
</table>
8. Irrigation

Irrigation of wasabi has two important functions:

(1) To maintain soil moisture level
(2) To assist cooling of the plants.

Weekly applications of 30 mm water is adequate for wasabi. If the soil becomes saturated the amount of oxygen available to the roots is reduced and their function and growth restricted. Allowing the soil to drain adequately will help to produce even growth and higher quality stems. If a hot day is predicted, irrigation should be applied in the morning before temperatures rise to help keep the plants cool during the heat of the day. Water applied over the shadehouse will help reduce the air temperature inside due to evaporative cooling.

Wasabi may be irrigated by overhead sprinklers, drippers or weeping hose provided the water is distributed evenly across the bed. Sprinklers with a small droplet size are preferred to prevent plant damage. If a permanent system is installed, care should be taken to prevent leakage from the sprinklers as this will cause a saturated area of soil and possible plant death through waterlogging and disease.
9. Pests and Diseases

Prevention, identification and management of disease is extremely important. Reports from Japan and New Zealand indicate that wasabi is susceptible to bacteria, fungi and viruses. Because wasabi is a new crop to Australia, a permit from the National Registration Authority (NRA) is required for any agricultural chemical used in protecting the crop. Temporary permits for specific products have been issued for use on Tasmanian wasabi crops. With the exception of products described below new growers should contact DPIWE before applying any agricultural chemicals to wasabi.

9.1 Diseases

**Fungi**

Fungal organisms readily invade wasabi plants which have been subject to stress (heat, water, nutritional) or physical damage. *Phoma lingam* and *Fusarium sp.* have been identified in Tasmanian wasabi crops. Treatment of fungal organisms during the summer months (November to March) is particularly important.

Products containing 400g/kg copper (eg. Kocide®) can be used as a protective fungicide against these two organisms. Products containing 500g/kg benomyl (eg.Benlate®) can be used to control outbreaks of *Phoma* and *Fusarium*. These products should be used at the rate recommended by the manufacturers for use on brassica vegetables.

Small outbreaks of white rust (*Albugo sp.*) have also been identified in Tasmanian wasabi crops. These have been controlled by removal of the affected leaves and their disposal away from the site.

9.2 Pests

**Aphids**

Aphids are sap-sucking and cause wilting and stunting of young plants and yellowing, puckering and curling of older leaves. They are usually found on the undersides of leaves in clusters or clumps most commonly in spring and autumn. Weekly monitoring of the crop will allow early detection of insect attack. Natural predators such as ladybird larvae, tiny wasps and hover flies can be protected by the use of 'safe' aphicides. The most common aphid identified to date is green peach aphid which is greenish pink in colour.

The NRA has issued a temporary permit for the use of products containing 500g/kg pirimicarb to treat aphids. This permit is valid until July 1, 2002. New growers should contact DPIWE after this date to clarify permit status.

The following insects affect other members of the brassica family (e.g. cabbage, cauliflower, broccoli, and brussels sprouts) and could cause damage in wasabi crops.
Cabbage white butterfly (Pieris rapae)

Occurrence: Cabbage white butterfly is most active in spring and autumn. Young caterpillars are found on the undersides of leaves and then move towards the centre of the plant. Older caterpillars are usually on the underside of the leaves.

Adult: A medium sized light grey or yellowish white butterfly with black tipped forewings with central black spots.

Eggs: Are visible to the naked eye and are pale yellow spindle shaped laid singly on the underside of leaves.

Grubs: Are velvety green and about 2.5 cm when mature. They have faint yellow stripes along the sides of the body.

Damage: Caterpillars rapidly chew large holes in the leaves.

Cabbage moth (Plutella xylostella)

Occurrence: Caterpillars can be found from early spring until early winter but are most abundant between January and May. The winter is passed in the moth stage. Moths fly and lay eggs at dusk but can be seen during the day if plants are disturbed.

Adult: A small (1 cm) active greyish brown moth with diamond shaped white markings on its back.

Eggs: Are just visible to the naked eye and are greenish yellow and disc shaped. Laid singly or in clusters usually lying close to a vein on the underside of leaves.

Grubs: The first caterpillars are very small and burrow into the underside leaf surface. They grow into 1 to 1.5 cm long bright green cigar shaped grubs which are very active, often using silken threads to move around.

Damage: Caterpillars first mine the leaves from the lower surface with older caterpillars removing all but the very upper surface leaving ragged patches and holes.

Light brown apple moth

Occurrence: These have been found in the glasshouse but are seldom seen in the field. Caterpillars are active from early spring to autumn.

Adult: A small (1 cm) light brown moth which hides in the foliage but can be seen when the foliage is disturbed.

Grubs: Very active greenish grey caterpillars which grow to about 1 cm in length. Often found at the top of the petiole where it joins the leaf blade.

Damage: Caterpillars chew into the plant tissue where the petiole joins the leaf blade causing the leaf to wilt, they then produce webbing which holds the two sides of the leaf together. Tissues of the underside of the leaf are chewed eventually creating holes.

White cabbage butterfly, cabbage moth and light brown apple moth can all be treated with DIPEL® (32,000 IU/mg *Bacillus thuringiensis var kustaki*).
Slugs and Snails

These most often occur when there is a lot of plant debris or clods around in moist conditions. Look for these at the base of plants and under soil clods. They can particularly damage at the soil level and allow diseases to enter the plant. Snail baits containing 20g/kg methiocarb or 15g/kg metaldehyde should be used if snails and slugs are present.

Rodents

Rats have been identified in Tasmanian wasabi particularly when the stems are sufficiently mature to harvest. Bait stations placed near the entrance to the rat hole are effective in controlling these pests.

Minimal use of agricultural chemicals in the production of Tasmanian wasabi is a marketing asset that all producers should strive to attain.
10. Propagation

10.1 Vegetative

Wasabi regenerates easily from vegetative side shoots. This is a suitable method of propagation from healthy, disease free plants. The number of side shoots from a 2 year old plant ranges from around 8 to 30. The main disadvantage is transfer of disease from the mother plant. Vegetative propagation is normally only carried out for 2 or 3 seasons to prevent disease build up.

10.2 Seed production

Wasabi sends out long flower stems in both autumn and spring from mature plants (about 1 year old). These are leafy and small white flowers appear at the tip. Wasabi requires cross-pollination and native flies are suitable insect vectors. Up to 8 seeds develop in long pods. Seeds are mature when the pods are fat and either green or purple in colour. When the pods at the base of the flower stalk begin to open and drop their seed the whole flower stalk can be harvested to prevent further seed falling to the ground.

Wasabi seed loses viability rapidly if allowed to dry out. Seed should be left in pods which can be collected and placed into plastic bags or wet sand and stored at 1 to 2°C before sowing. Seed produced from plants flowering in autumn have greater viability than those produced from the spring flowering. Treatments which have been found to improve seed germination percentages include:

(i) Flushing whole pods in cool (12 to 15°C) running water for 12 months. Flushing also helps breakdown the pods, making the seed easier to remove.

(ii) Chilling pods in wet sand for 42 days.

(iii) Treating seed with 400 ppm gibberellic acid for 72 hours prior to sowing.

Wasabi seed can then be planted in flat trays in a soil-less medium such as peat:sand:perlite and maintained at around 15°C. The young seedlings are then pricked out into cell trays and are ready for transplanting about 3 months from sowing.
11. Harvest

Individual stems of wasabi which have reached marketable size can be harvested or the whole plant can be harvested and stems graded as:

(i) Premium stems suitable for the fresh market. These are valued at $100/kg on the domestic market.
(ii) Smaller stems suitable for processing as prepared wasabi paste or dried powder. These are valued at $20/kg on the domestic market.

(See section on presentation below).

The largest stems usually reach a marketable size of 8 to 15 cm long and weigh 60 to 150g two years after planting. From 4 to 14 stems suitable for the fresh market may be found on an individual plant. Wasabi will develop numerous side shoots following flowering so it is best to harvest stems before these enlarge.

12. Presentation

12.1 Colour

Generally the outside of the stem of Japanese wasabi is bright green. Wasabi that is either light (yellow-green) or dark (brown-green) is considered inferior though still marketable. One of the most important aspects of colour is that the inside of the stem should be pale green to ensure a good coloured paste when the stem is ground.

12.2 Shape

Wasabi should have an evenly tapered stem. Uneven taper indicates that the wasabi has been subject to stress imposed by variation in environmental conditions during the growth period.

12.3 Trimming and Cleaning

Trimming is a critical part of wasabi stem presentation. Excessive trimming indicates that soil staining had to be removed or there was disease present, while insufficient trimming devalues the product (e.g. by including lower value petiole or roots). High value wasabi stems have the roots removed but the base of the stem is left intact. Petiole trimming is also important. Generally petioles should be trimmed evenly to a length approximately one third the length of the stem. Wasabi stems may be cleaned under cool water using a soft brush. There should be no trace of soil particles or staining on the clean stems. Cleaning of wasabi stems with water under high pressure is fast and effective though care must be taken to prevent damage to the soft tissue of the petiole.

Stems should be kept moist, cooled to 4°C immediately after harvest, packaged in chilled polystyrene boxes and delivered to the market within 24 hours.
Wasabi stems cleaned and trimmed
12.4 Weight
Generally stems weighing from 60 to 180g are preferred for the fresh market. Processing grade stems are those weighing less than 60g or that are bent or broken.

12.5 Disease
Stems should be free from disease both internally and externally.

12.6 Leaves
The large dark green leaves of wasabi are also sought by the fresh market. Leaves 100 to 150 mm in diameter and which show no discolouration or physical damage are used in presentation of Japanese food and are a tasty and attractive addition to fresh salads. A price of $5 for a bunch of 10 leaves has been attained on domestic markets.

Leaves should be harvested early in the morning and packaged in breathable plastic bags cooled to 4°C and delivered to the market within 24 hours.

13. Yield
A yield of 10 t/ha has been recorded from the first semi-commercial crops of Tasmanian wasabi. Potential to double the yield is high once varietal selection and improvements in crop husbandry have been addressed.

Seed from variety 'Daruma' developed many plants with multiple stems too small to market. In addition blackened lesions on some stems made them unsuitable for market. The blackened lesions are thought to be caused by a secondary fungal infection induced by plant stress.
8.3 Appendix 3  Wasabi Market Research Summary

Methodology

A sample of four wasabi stems, clearly numbered (1 to 4) were chilled packed and posted to each of
the various market contacts in Hobart, Launceston, Melbourne and Sydney, a day after stems were
harvested. Each stem in the samples was measured, weighed and recorded prior packing.

Telephone surveys were conducted by the same person (Nani Clark) over a few consecutive days, after
packed samples were courier delivered to market contacts. Research was conducted only with the end
user or key decision maker/purchaser of the wasabi. Each interview took 45mins to an hour, a copy of
survey is attached.

Survey Results

Appearance:
- Needs to be a bit greener.
- Good.
- Very important to have diameter of stem uniform.

Taste:
- Fantastic, improved a lot.
- Needs a stronger rich balanced wasabi flavour, longer lasting taste, a breadth of heat and
sweetness.
  (Cannot be stringy or powdery, the older the plant (4-5 years), the better the wasabi taste)

Ideal Stem:
No. 1, Batch 3 (145mm by 28 mm, 110g)
No. 4, Batch 5 (83mm by 21mm, 45g)
No. 4, Batch 1 (80mm by 22mm, 65 g)
No. 4, Batch 6 (70mm by 24mm, 45g)

Ideal Length and Size of Stem:
- 70mm by 15mm
- 80-95mm by 19-23mm, 60-65g
- 100mm by 28mm, 100g
- 180–240mm by 33-45mm, 135-172.5g.

Products:
1) Fresh premium stems
2) Freshly minced, packed in tubes or jars for convenience and less wastage. (But must be able to
retain the original grated wasabi flavour)
3) Frozen wasabi
4) Wasabi Leaves fresh or pickled in saki kasu (rice wine)

Price:
$10/stem ($2.50, $6.50 - $8, >$10, $20-$25, $25-$30)
Packaging:
- Loose
- Individually packed, not squashed.
- Chilled packed with moisture/absorbent paper
- Clear labels with proof of country of origin, proof of harvest
- Quality assurance: Wasabi needs to always arrive in the same quality condition. (Stems not dried out, nor are stems allowed to sweat and thus rot.)
- In uniquely designed packages e.g.
  - Stems packed with a grater; with other seafood packs
  - Stems individually wrapped with pine leaves or seaweed.

Target Market:
- Higher end of Japanese restaurants in cosmopolitan areas, not sushi bars or Japanese Takeaways. Estimated Quantity Demand: 4 stems for smaller restaurant/week, 1 to 2 dozen stems for large restaurant/week.
- Export to Japan high end markets, quality restaurants and hotels.

Marketing Images:
- Beautiful, green, uniformed wasabi stems and freshly grated wasabi served with sashimi and sushi.
- Tokyo fish market in January.

Marketing messages:
- Naturally grown, without any pesticides and chemicals, no artificial additives.
- Grown in Tasmania, the island state with one of world’s cleanest water, air and land. Crystal clean running water/rivers.
- Best served with raw fish and sushi.
- Umami flavour.
- A beautiful aromatic taste, depth of combination in natural heat and sweetness.

Opportunity and Threats:
As most wasabi purchase agreements are casual, it is easy to enter market to supply product (Opportunity). On the flip side, it also means that threats of new entrants and other potential competitors into our market is high.

Many Japanese believe that wasabi can kill certain bacteria found in raw fish. (Opportunity)

Competitors:
Korean wasabi quality is not consistent, sometimes can be quite good and sometimes not as good. (But never as good as premium Japanese water wasabi.)
New Zealand Wasabi (2 years ago) did not have very much wasabi aroma or flavour.

Buyers' Readiness to Purchase:
- Definitely.
- Definitely. Depending on price and consistency in supply.
- Definitely, if quality improves and depending on the price.
- Maybe, depending on price, quality and consistency in supply

Key Buying Reason:
- Essential condiment for raw fish, sea-urchins
- Enhances the flavour of seafood
- Believe to kill certain bacteria in raw foods
**Critical Factors in Determining Purchase:**

1) Quality
2) Taste and flavour. Balanced and long lasting.
3) Hot smell and heat.
4) Size
5) Price

**Critical Factors in Production:**

1) Quality Assurance. Ability to supply consistency in quality of appearance and taste. Ensure stems are good on the inside as on the outside, not black when cut. Ensure that the wasabi always has a strong taste, not sometimes tasteless, sometimes bitter, or stringy, or powdery, no musty smell.

2) Producing greener stems.

3) Producing stems that have longer lasting flavour ie. the taste lingers for a while in the mouth and not to have the taste of wasabi change as rapidly after grating. (Japanese wasabi can be grated and stored in an airtight container for a few hours without having a change in its flavour. Whereas Tasmanian wasabi can lose its flavour upon minutes after grating). Producing wasabi with stronger taste, a deep combination of natural sweetness and heat.

4) Producing more uniformed stems, not knobbly.
What is your occupation?

What is your cultural background?

How would you grade the quality of this wasabi?

**Colour**
1: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)
2: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)
3: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)
4: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)

**Aroma (Before and after grating)**
1: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)
2: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)
3: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)
4: Poor 1…2….3….4….5……6……7……8……9……10 Superb (Circle One)

**Texture (On the tongue, smooth? Rough?)**
1: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)
2: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)
3: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)
4: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)

**Taste** (with soya sauce) – Strong wasabi flavour? Umami? Long lasting taste? Sweet and hot taste at the same time? Bitter?
1: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)
2: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)
3: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)
4: Poor 1….2….3….4….5……6……7……8……9……10 Superb (Circle One)

**Appearance**
1: Poor 1….2….3….4….5….6….7….8….9….10 Superb (Circle One)
2: Poor 1….2….3….4….5….6….7….8….9….10 Superb (Circle One)
3: Poor 1….2….3….4….5….6….7….8….9….10 Superb (Circle One)
4: Poor 1….2….3….4….5….6….7….8….9….10 Superb (Circle One)

Would you purchase this wasabi? (1, 2, 3, 4)
☐ Definitely ☐ No ☐ Maybe

If no, why not?
☐ Not the right quality ☐ Not the right form ☐ Not hot enough
☐ Other (Please specify) ________________________________

If maybe, what will purchase be dependent on?
☐ Price ☐ Consistent supply ☐ Current supplier arrangement
☐ Other (Please specify) ________________________________

How much will you pay for this wasabi? (Price for top grade fresh, 2nd grade fresh and processed)

How much will you purchase? (Quantity and frequency)

Where are you currently purchasing your wasabi?

What sort of purchase agreement do you have with your current wasabi supplier?

What would be an ideal purchase arrangement? (How you would like to purchase wasabi? Would you want your wasabi to be delivered and how often?)

In what forms are your wasabi needs?
☐ Dried powder ☐ Paste ☐ Fresh
Which form of wasabi would you consume the most?
☐ Fresh  ☐ Dried Powder  ☐ Paste  ☐ Minced  ☐ Other ____________
Why? __________________________________________________________________________

Which form of wasabi is your favourite?
☐ Fresh  ☐ Paste  ☐ Powdered  ☐ Frozen  ☐ Other ____________
Why? __________________________________________________________________________

What is your favourite dish with wasabi?
________________________________________________________________________________

How much wasabi are you currently buying?
________________________________________________________________________________

What do you think your demand for wasabi is going to be in the near future?
☐ Increase greatly  ☐ Increase moderately  ☐ Increase slightly  ☐ Unchange
☐ Decrease slightly  ☐ Decrease moderately  ☐ Decrease dramatically

When you think of wasabi, what is the first thing that comes to mind?
________________________________________________________________________________

When you think of quality wasabi, what comes to mind immediately?
________________________________________________________________________________

Which country’s wasabi would you pay the highest premium? Why or why not?
(Are there particular attributes of wasabi for which you would pay a premium?)
________________________________________________________________________________
Describe the perfect wasabi.
Taste:
____________________________________________
____________________________________________
____________________________________________
____________________________________________
Texture:________________________________________
Smell:________________________________________
Colour:________________________________________
Length:________________________________________
Shape:________________________________________
Size:________________________________________

What is most important factor to you in terms of your wasabi? Why do you eat wasabi?
______________________________________________
______________________________________________
______________________________________________

What is the most important decision making factor to you in terms of your purchase of wasabi?
______________________________________________
______________________________________________
______________________________________________

What other forms of wasabi and wasabi products would you demand?
☐ Pickled wasabi  ☐ Wasabi Leaves  ☐ Minced  ☐ Freeze dried
☐ Other _______________________________________

How would you want your wasabi to be presented?
☐ Fresh  ☐ Loose  ☐ Vacuum packed  ☐ Chilled packed  ☐ Individually packed
☐ Other _______________________________________

☐ Processed:  ☐ In a paste-tube  ☐ In a jar
☐ Other _______________________________________

8.5 Appendix 5  Chromatographic analysis of wasabi stems
Further to our phone conversation the other day, I have attached a chromatogram of the volatiles found in the freshly cut wasabi rhizome, with peaks annotated. This analysis was done by Solid Phase Micro Extraction (SPME), in which compounds in the vapour phase are adsorbed into a needle, which is then thermally desorbed and analysed by combined gas chromatography-mass spectrometry (GC-MS). During the course of the analyses, I found there was a difference in the results depending on the injection temperature used. Further researching of the literature indicated that allyl isothiocyanate, the reported main volatile constituent of freshly ground wasabi, can be converted to allyl thiocyanate with heat, and I confirmed that only a single peak was observed when much lower injection temperatures were used. However, as these low temperatures compromised the desorption of the less volatile components, I have had to include the chromatogram from the higher temperature runs, and marked the putative allyl thiocyanate as an artefact. Further investigation would be necessary to thoroughly explore this difficulty.

Many of the listed components of wasabi were detected, plus at least one that does not appear in the Depree paper. No methylthioalkyl isothiocyanates have yet been identified, but the failure to detect these compounds does not necessarily imply they are not present it could be that the SPME approach is unsuitable to detect them, or they are present only in the distilled oil rather than freshly cut tissue, or that they are present in amounts too low to be seen by this analytical approach. I don't have reference MS data for all of the expected isothiocyanates and so some of these have been assigned based on interpretation of the mass spectra from first principles, in the context of the known wasabi components. I also as yet have no reference MS data for any of the methylthioalkyl isothiocyanates, which may be available in some of the Japanese wasabi literature.
The following compounds were detected, with approximate proportions indicated by the relative areas of the peaks shown in the attached chromatogram.

- isopropyl isothiocyanate
- allyl isothiocyanate
- sec-butyl isothiocyanate
- isobutyl isothiocyanate
- 3-butenyl isothiocyanate
- unidentified C₅H₉NS compound
- a pentyl isothiocyanate (unidentified isomer, not listed in Depree reference)
- 4-pentenyl isothiocyanate
- unidentified C₆H₁₁NS compound
- 5-hexenyl isothiocyanate

Further work, and access to additional literature on wasabi components, will be necessary for a more detailed breakdown of composition.

[Signature]

Officer-in-Charge, Organic Mass Spectrometry

Allyl
n-Butyl
3-Butenyl
4-Pentenyl
5-Hexenyl
2-Phenylethyl
5-Methylthiopentyl
6-Methylthiohexyl
7-Methylthioheptyl
5-Methylsulphinylpentyl
6-Methylsulphinylhexyl
7-Methylsulphinylheptyl
8.6 Appendix 6  

Appendix 6.1 'Wasabi Tasmania'

Information sheet prepared by DPIWE at 'Taste the harvest' Food and Wine Festival, Devonport, Tasmania, April 2000.

Wasabi Tasmania

Wasabi (Wasabia japonica) is a Japanese brassica vegetable, the stems of which are used to prepare a hot-tasting condiment traditionally served with sushi, sashimi and soba noodles. Reduction of the area suitable for the cultivation of this plant in Japan and the aging population of the traditional farmers has lead to a decline in the availability of wasabi. The market however continues to expand as both Japanese and cross cultural enthusiasts recognise the qualities of this product.

Tasmania has been identified as a state where cool climate vegetables will thrive and can be made available to Asian markets in the off season. For this reason wasabi has been trialed using the combined resources of RIRDC and the Department of Primary Industries, Water and Environment, in collaboration with local growers.

Wasabi is a semi-aquatic plant which can be grown in gravel beds adjacent to cool flowing streams or in well-drained soils which provide good root aeration.

Soil grown wasabi crops have been established in shade houses at two sites in North Eastern Tasmania (each about 0.2 ha). These crops are now approaching maturation at 2 years of age and will be harvested in the winter of 2000. Yield estimates are in the range of 5 tonnes/ha of premium stems suitable for the fresh market and an additional 15 tonnes/ha of stems suitable for processing to produce wasabi paste and powder.

Trials conducted to determine optimum conditions for germination have given vital information regarding storage and handling of wasabi seed and seedlings.

Continued interest from the private sector regarding the availability of both the marketable product and plant material for production purposes has lead to the involvement of a Tasmanian tissue culture laboratory in the propagation process. Initiation material will come from parent plots of wasabi which have been selected on the basis of their superior stem characteristics.

Preliminary investigations into the market potential of wasabi has initiated great enthusiasm from prospective buyers. At present, priority is being given to ensuring the continued supply to the market of a product of superior quality.

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Appendix 6.2  ‘Wasabi Production in Tasmania’.

Information sheet prepared by DPIWE presented at 'Alternatives in Agriculture' forum Launceston, Tasmania, November 2000.

WASABI PRODUCTION IN TASMANIA

Introduction

Wasabi or Japanese horseradish (*Wasabia japonica* Matsum), a perennial herb, grows naturally alongside mountain streams in Japan. It is an established condiment with traditional Japanese food including soba, sushi and sashimi. The high prices received for fresh wasabi, up to $100/kg, and its requirement for cool growing conditions prompted the introduction of the crop to Tasmania for commercial evaluation in 1992.

Propagation

Of the varieties used in trials ‘Daruma’ was found to be best suited to Tasmanian conditions.

Seed production

Flowering and seed set takes place twice a year in late spring and autumn. The normal habitat for wasabi is beside mountain streams where the sticky seeds are released into a moist environment and after a period of cold incubation the seeds are able to germinate.

Characteristics of wasabi seed make storage and handling requirements unusual. The seed does not survive desiccation nor low oxygen levels. It has a vernalisation requirement of 28 days below 4°C after which time it will germinate at this temperature. Warmer storage temperatures increase its susceptibility to fungal contamination.

Vegetative

Wasabi regenerates readily from side shoots. To maintain a disease-free population, this method of propagation is recommended for a maximum of 2 generations.

Tissue culture

This technique is currently being investigated by the Tasmanian Institute of Agricultural Research and a commercial laboratory in Launceston, so that selected strains of wasabi can be rapidly multiplied and made available to interested growers.
Production systems

Wasabi grows naturally under the shade of deciduous trees. A shade-house providing 80% shade will provide optimum light intensity conditions and shelter from the wind.

Semi-aquatic

Semi-aquatic cultivation, referred to as ‘mizu’ in Japan, has a reputation for producing large, high quality, premium priced stems. The plants are grown in gravel beds through which water permeates.

Potential exists to establish semi-aquatic wasabi in Tasmania at sites where cool, running, water can be provided. Ideal water temperatures range from 12-15°C and good aeration is essential. Air temperatures should not exceed 30°C. Trial sites downstream from freshwater fish farms and hydroponic systems show potential for development.

Soil culture

Extreme air temperatures and soil type are the major limitations to soil cultured wasabi. Optimal air temperatures range from minus 3 to 30°C and soil temperatures from 12 to 15°C. Outside this range growth is slowed and stems produced are not uniform.

Wasabi grows most successfully in soils which are free draining such as krasnozem or sandy loam. Addition of fertilisers at rates similar to those of other brassica vegetables is recommended with regular side dressings of nitrogenous fertiliser. Foliar applications of boron are recommended.

Pests and Diseases

The low temperature at which wasabi grows results in low incidence of pests and diseases. Populations of predatory slugs and snails can be reduced with the use of an organic mulch such as sawdust, wood chips or barley straw around the shade house or by early establishment of prostrate rosemary bushes around the perimeter of the site. Applications of a deterrent containing metaldehyde or methiocarb are may be required in addition to these preventative measures.

The incidence of blackened lesions in some mature stems has been attributed to the fungus *Phoma lingum*. This disease can be controlled by using copper sprays such as Kocide® or sprays containing benomyl such as Benlate®.

Yield and Quality

The harvest of a semi-commercial crop of wasabi produced in soil culture commenced in the winter of 2000. The product is of high quality and initial market tests have met with an enthusiastic response. Yield estimates are in the order of 10 tonnes/ha. This includes the high priced premium stems for the fresh market ($100/kg) and secondary stems suitable for processing. The average market value of the crop is estimated at $32/kg.

Contact

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Appendix 6.3 'Tasmanian Wasabi'

Brochure prepared by Department of Primary Industries, Water and Environment, presented at 'Taste the Harvest' Food and Wine Festival, Devonport Tasmania, March 2001.

Tasmanian Wasabi

Wasabi (*Wasabia japonica*) is a Japanese brassica vegetable, the stems of which are used to prepare a hot-tasting condiment traditionally served with sushi, raw fish and soba noodles.

Tasmania has been identified as a state where cool climate vegetables will thrive and can be made available to Asian markets in the off season. For this reason, wasabi has been trialled using the combined resources of RIRDC and the Department of Primary Industries, Water and Environment, in collaboration with local growers.

The Tasmanian government together with local producers have employed a Horticulturist to conduct trials on wasabi since 1991. Trials conducted to determine optimum conditions for germination have given vital information regarding storage and handling of wasabi seed and seedlings.

Continued interest from the private sector regarding the availability of both the marketable product and plant material for production purposes has lead to the involvement of a Tasmanian tissue culture laboratory in the propagation process. Initiation material will come from parent plots of wasabi which have been selected on the basis of their superior stem characteristics.

Our crop is destined for the domestic market. Market research indicates that the quality of our product is suitable for the fresh premium market, largely dominated by the restaurant trade in Australia. In addition to these buyers, processed material will be sold to companies involved with companion products, e.g. with fish dishes and soft cheese. We are now confident that the quality of our wasabi is sufficient to engage the interest of our South-East Asian neighbours. We would appreciate any market indicators which would enable us to plan for further expansion of production here in Tasmania.

![Wasabi grown under shade at Winnaleah, at I.R. Farquhar's.](image)

Japanese Ambassador, His Excellency, Mr Masaji Takahashi and his wife Madame, Mrs Yuri Takahashi were very impressed with quality of Tasmania wasabi at our tasting in April 2000.

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8.7 Appendix 7  Media articles

- 'Come to grips with wasabi' Sunday Tasmanian p. 28 November 12, 2000.
- 'Wasabi proves a hot seller' by Fran Voss, The Examiner p. 3 August 30, 2000.
- 'Agora' the in flight magazine for Business Class travellers with Japan Airlines Company Limited, Autumn 2001 issue.
- 'Australian Gourmet Traveller' May-September 2001 issue (in Press).

Full copies of these articles have been submitted as hard copy, but are not available electronically.
9. Publications


10. Glossary

Dehisce: to burst open; natural splitting of seed pods to liberate seeds.
Desiccation: becoming dry by removal of water
Raceme: a branched indeterminate inflorescence, flowers are borne along the length of the branched flower stalk.
Rhizome: above ground stem, the buds may give rise to side shoots or roots.
Vernalisation: a chilling treatment required to break dormancy or initiate flowering.
Elutriation: separation of lighter and heavier particles in a mixture by washing or gas flow.
11. References


