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Morphological and Chemical Evaluation of *Atractylodes lancea* Plants Raised from Refrigerated Shoot Cultures

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Plant micropropagation through shoot tip culture is routinely employed in the field of agriculture, especially in horticulture, these days. At the various stages of micropropagation, there is a need for storage of the cultures, *e. g.*, maintenance of stook cultures, temporal storage of cultures due to seasonal factors and so on. Slow growth achieved simply by a reduced temperature is a practical method for medium-term storage of shoot cultures, since this method thought not to cause morphological variation in plants[1]. It seems, however, that experimental evidence for biochemical stability of plants raised from refrigerated shoot cultures is lacking. In the present study, we field-tested the plants derived from refrigerated shoot cultures of *Atractylodes lancea* DC., a perennial medicinal herb of the family Asteraceae, for stability of secondary metabolite accumulation. The metabolites analyzed are two sesquiterpenes (hinesol, β-eudesmol) and an acetylenic compound (atractylodin) which are contained as major components of essential oil in the rhizome.

Shoot cultures were started from shoot tips of a single plant of *A. lancea.* The micropropagation[2,3] and refrigerated storage[4] of *in vitro* shoots were carried out as described previously. The shoot cultures of the 35th generation were stored at 2 °C in the light (16h/day, 500 lux) for 24 months following preincubation on Linsmaier-Skoog medium[5] containing 1 μM 6-benzylaminopurine for 14 days at 25 °C under light irradiation (16 h/day, 2000 lux). After storage, these refrigerated shoots were cultured on the shoot proliferation medium at 25 °C under the cycling liht (2, 000 lux) for three consecutive subcultures at intervals of 1 month. The control shoot cultures were maintained by regular subculture without cold storage. The control shoots of the 62nd generation and the cold-treated shoots of the fourth generaiton after storage were transferred at the same time onto hormone-free Linsmaier-Skoog medium for rooting. Both the groups of plantlets were potted, acclimatized in mist for 2 weeks, and kept for further 6 weeks on the shelves in the greenhouse. Finally they were transplanted to the experimental field on November 7, 1990 and harvested on September 28, 1992. Morphological characteristics of the harvested plants shown in **Table 1** were recorded. Rhizomes were dried in a hot-air oven at 50 °C for 3 days. Hinesol, β-eudesmol and atractylodin contents in the rhizome were determined by the gas liquid chromatographic method reported earlier[4].

A. lancea shoot cultures can survive perfectly cold storage at 0-5 °C for up to 24 months[4]. In our continuous study, the survival rate of shoots decreased to about 50% after 40 months of refrigerated storage and the surviving shoots grew normally to plantlets. In the present study, we used the shoot cultures cold-stored for 24 months. To assess the after-effects of storage we field-tested the plants

Table 1. Morphological characteristics and contents of essential oil components in the rhizome of field-cultivated *Atractylodes lancea* plants raised from refrigerated shoot cultures.

Characteristic	Control plants (n=34)			Plants raised from refrigerated shoots (n=21)		
	Mean	SD	CV(%)	Mean	SD	CV(%)
Plant height (cm)	23.5	5.2	22	22.1	5.9	27
No. of capitula per plant	1.6	1.4	88	2.2	1.8	82
No. of stems	1.7	0.8	47	1.6	0.9	56
No. of nodes in the longest stem	16.2	2.5	15	18.9*	2.5	13
Fresh wt. of rhizome (g)	6.0	2.8	47	6.2	3.1	50
Dry wt. of rhizome (g)	1.8	0.8	44	1.9	0.8	42
No. of roots	20.6	9.3	45	24.2	7.5	31
Hinesol content (% of dry wt.)	0.10	0.06	60	0.12	0.08	67
β-Eudesmol content (% of dry wt.)	0.25	0.13	52	0.24	0.13	54
Atractylodin content (% of dry wt.)	0.21	0.04	19	0.23	0.06	26

* Significantly different from the control by Student's t-test, $p<0.05$

derived from the refrigerated shoots. The survival rate of the test and control plants in the field was 97 (31/32) and 80% (70/87) and the bolting rate 77 (24/32) and 76% (53/87), respectively, at the harvest time. **Table 1** shows the results of the field trials. There were no significant differences in morphological features between the two groups except the number of nodes. Coefficients of variation of each characteristic were similar between two groups. No abnormalities were observed in the morphological traits of the test clonal plants such as leaf shape, flower shape, and color. The respective contents of hinesol, β-eudesmol and atractylodin in the rhizome were not significantly different between two groups (**Table 1**). The present field trials clearly indicate that refrigerated storage does not cause adverse after-effects on morphology and the secondary metabolite accumulation of field-cultivated plants raised from refrigerated shoots. It is concluded that refrigerated storage of cultured shoots is an effective and dependable method for preserving both the morphological and chemical characterisics of *A. lancea*.

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《和文要約》

冷蔵培養シュートから育成したホソバオケラの形態学的並びに化学的評価

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ホソバオケラ（キク科）の培養シュートをシュート増殖培地上で2年間冷蔵（2 ℃）保存した後，増殖，発根，鉢上げして，2年間圃場栽培した植物の形態学的形質および根茎中の hinesol, β-eudesmol, atractylodin 含量について検討した．その結果，冷蔵処理ショート由来の植物と未処理で継代培養したシュート由来の対照植物には有意差がなかった．