Jour. agric. Res. China (中華農業研究) 30(4) : 369~377 (1981)

Low temperature treatment and flower development in Ta-Mei-Fua radish[1]

Tzay Fa Sheen[2]

Abstract : A seed vernalizing type of radish was used to find a method to promote flowering in late bolting radish for breeding in the tropics. The relationship between morphological changes of the shoot apex and metabolic changes in nitrogen, carbohydrates compounds during flower development was also investigated.

Results indicated that mature Ta-Mei-Fua radish will flower when defoliated of top parts and the root is exposed to 9±1°C temperatures and 16 hr daylight for three weeks.

The number of tunica layers on the shoot apex increased from 4-5 layers to 5-6 layers when mature plants were exposed to three weeks of low temperature treatment. The number of layers decreased rapidly to three layers when mature plants transited from the generative stage to the reproductive stage. At the same time, cell shapes on the corpus layers changed from their original flat type to an erect swollen type. The flower primordium began to form after four weeks of low temperature treatment. Sugar content increased during low temperature treatments and was highest when cell division on the shoot apex was at a maximum and the tunica layers were increasing to 5-6 layers. Sugar content gradually decreased as the number of tunica layers decreased to three and flower primordium formation gave way to bolting. Changes in sugar contents were similar to those of mature green vernalization type crops when treated with low temperatures.

Cultivar Ta-Mei-Fua is one of the major radish cultivars planted during the autumn and spring cropping seasons in Taiwan. When this crop is planted in northern Taiwan or at high altitudes, low temperatures cause premature bolting, reducing both yields and quality for fresh market production. Seed production, on the other hand, suffers from insufficiently low temperatures to induce bolting and flowering of selected seed mother plants. Therefore, methods for preventing premature bolting of an economic crop in early or late winter and artifically inducing flowering of the seed mother plant for breeding work need to be devised.

1. Contribution No. 1015 from the Taiwan Agricultural Research Institute.

2. Senior Horticulturist and Director, Fangshan Tropical Horticultural Experiment Station, TARI, Fengshan City , Kaohsiung Hsien , Taiwan 830, ROC.

Radish is a seed vernalization type crop. A number of studies have indicated that low temperature treatments on germinating seeds are effective in accelerating flowering in radish. Yamato [20] showed a relationship between low temperature and the flowering response of a germinating radish seed. Eguchi [2] demonstrated that radish flowering was promoted when germinating seeds were exposed to 5°C temperatures for 15-20 days. Kagawa [6] showed that germinating seeds treated with low temperatures and sufficient light had a better flowering response than seeds treated with low temperature under total darkness. Sugiyama [17] showed differences in the flowering habits of several radish varieties and used these differences to classify the radishes. Shinohara [16] examined the genealogical and population genetic aspects of phasic development and classified the climatypes of radish. While these research on flowering physiology and morphogenesis of radish may also be applicable to the tropics and subtropics, no methods have been devised to promote flowering of selected seed mother plants under warm temperature conditions.

Seed mother plants were therefore selected to evaluate radish flowering behavior. All selected radish plants were exposed to low temperature conditions. Results from preliminary experiments indicated that radish roots appeared to vernalize efficiently when packed in transparent plastic bags and expose to light and longer low temperature treatments. The experiment investigated the morphological changes in the shoot apex and changes in nitrogen and carbohydrate compounds in the root during flower development. The morphological and histochemical changes of the mature root of a seed vernalizing plant type was also studied.

Materials and Methods

Radish cv. Ta-Mei-Fua was sown on October 17, 1980 and harvested on November 25, 1980. Plants were selected for the experiment at harvest according to size and uniformity. The leaf blades of all selected plants were cut to 1/3 height, and then 2-3 plants were packed together in 0.02mm transparent plastic bags for treatments at 9±1°C and 16 hr daylight. Each week, 25 plants were moved from the cold room. Twenty of these were transplanted to the field for observations on flowering response and the remaining five plants were used for analysis. This work continued every week for four weeks. A check plot was maintained in the field. On January 5, 1981, individual plants from each treatment were taken as samples. For all samples the shoot tip was fixed in a solution of formalin, alcohol and acetic acid (1 : 8 : 1). After dehydration and paraffin embeded, the tissue samples sliced into 12-15mm sections and stained with Ehrlich's Hematoxylin for anatomical observation. Root parts were dried in the oven at 60°C for nitrogen and carbohydrate analysis. Nitrogen was analyzed by the Kieldahl method. Soluble sugar and starch were analyzed by the Anthrone method and reducing sugar by the Somogi method. A flow chart of the experimental design is shown in Figure 1.

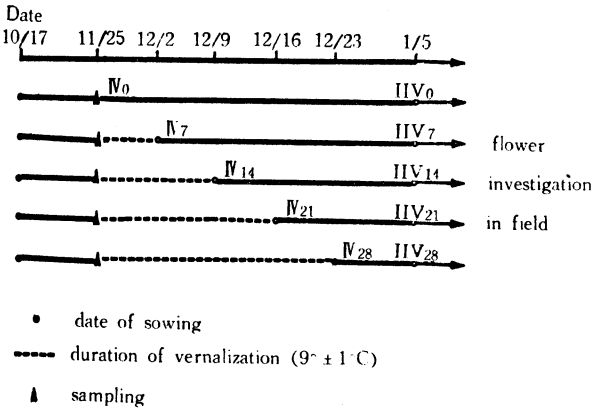


Fig. 1. Experimental plan for vernalization Ta-Mei-Fua radish.

Results and Discussion

Changes in the dry matter content the radish root after being exposed to low temperature treatments and transplanted to the field are shown in Table 1. Although new leaves continued to grow during the low temperature treatment, dry matter of the treated plots was less than that of the check, probably due to insufficient light exposure and lack of nutrient supply. Dry matter accumulation increased, however, after transplanting to the field.

Table 1. Dry matter (%) of radish root after vernalization

Treatment	%	Treatment	%
IV 0	5.9	IIV 0	5.5
IV 7	5.1	IIV 7	5.8
IV 14	5.3	IIV 14	6.4
IV 21	5.3	IIV 21	6.4
IV 28	5.5	IIV 28	6.2

Table 2. Changes in tunica layers of the shoot apex after low temperature treatment

Treatment	No. of plants examined	No. of tunica layers			Plants of floral primordia initated
		4-5	5-6	3	
IV 0	5	5			0
IV 7	5	4	1		0
IV 14	5	3	2		0
IV 21	5	1	3	1	0
IV 28	5		3	2	1

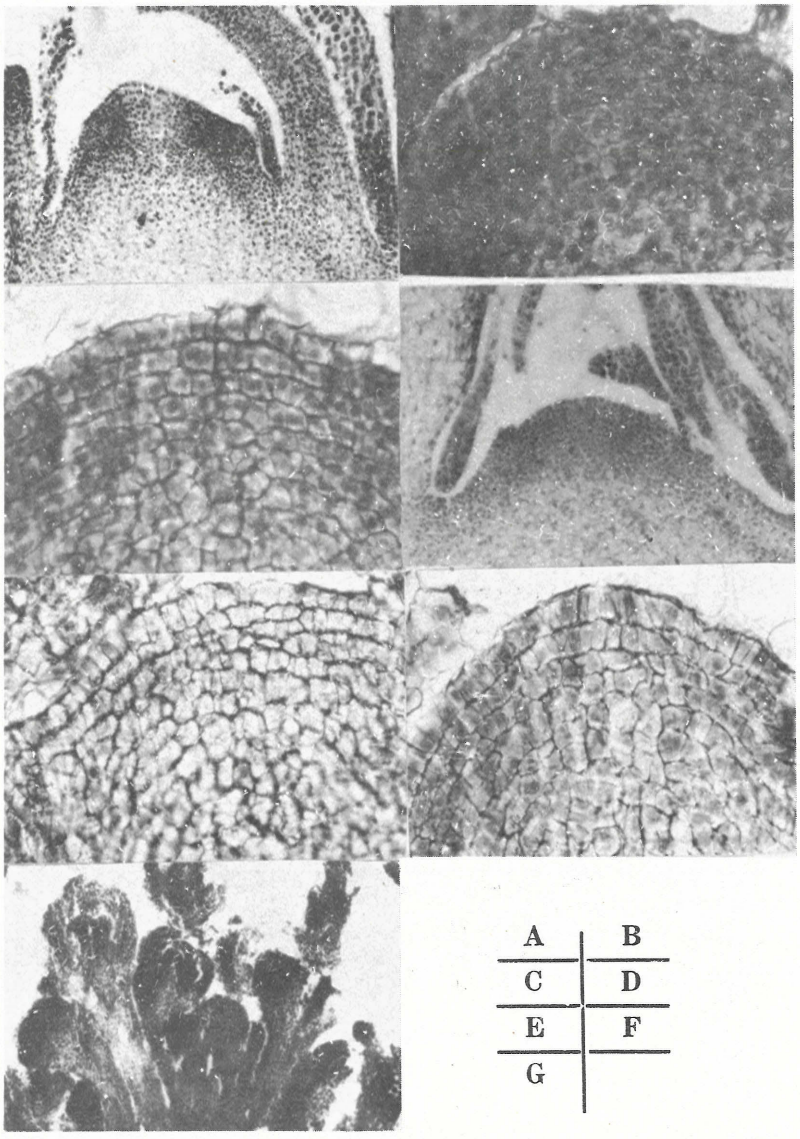


Fig. 2. Floral development of radish root treated with different durations of low temperatures. See Figure 1 for reference to the experimental plan.

A. Non-treated (IV 0) —Vegetative adult. 4—5 Tunica layers were observed. **B.** One week treated. (IV 7)—Vegetative stage is maintained; 4—5 tunica layers were observed. The height of the apex increased and the top grew ellipsoid. **C.** Two weeks treated (IV 14)—The apex gradully grew taller and thicker. 5 tunica layers were observed. Cell division increased on the upper part of the corpus. **D.** Three weeks treated (IV 21)-6 tunica layers were observed. **E.** Four weeks treated (IV 28)-The tunica layers decreased to 3, the shoot apex was more elongated. The first floral primordium was initiated at the inner part of the apex. **F.** Observation on January 5 of the one week treatment (IIV 7).—The tunica layers decreased to 3 and many new cells accumulated on the upper part of the corpus. **G.** Observation on January 5 of the four week treatment (IIV 28)-Flower bud and sepal were formed.

Table 2 and Figure 2 show changes in the tunica layer of the shoot apex after low temperature treatments. The shoot apex growth of the check plants appeared flat and contained 4-5 tunica layers (IVO). After one week of low temperature treatments the shoot apex appeared oblong and still maintained 4-5 tunica layers (IV7). The height and thickness of the shoot apex increased after two weeks of low temperature treatments. The number of tunica layers increased to 5-6, and more cell division activity appeared on the growing point with many new cells accumulating on the upper part of the corpus layer. The tunica layers then entered a transitional phase (IV14, IV21). The shoot apex appeared taller and tunica layers decreased from the original 5-6 dayers to 3 layers after 3-4 weeks of low temperature treatments. At the same time, the cells of the corpus layer assumed a more swollen shape (IV21, IV28). Flower primordia were initiated on the shoot apex of one plant after four weeks of low temperature treatments (IV28). Plants planted to the field on January 5, 1981 after one week or two weeks of low temperature treatments showed some flower primodium differentiation, but the plants exposed to four weeks of low temperatures were already bolting (IIV28). Figure 3 illustrates the effects of different durations of low temperature treatments on flowering behavior. The plants exposed to low temperatures for three weeks were the first to flower on February 8. All of the low temperature treatments had at least some plants that flowered, while the non-treated check had no flowering plants. The plants exposed to low temperatures for three weeks demonstrated the fastest flowering rate.

Table 3 shows the changes in nitrogen and carbohydrate contents of radish roots. Total nitrogen did not change significantly, but soluble nitrogen tended to increase with peak after the first week of low temperature treatment. Accordingly, insoluble nitrogen tended to decrease, reaching a nadir after the first week. Total carbohydrates, total sugar, non-reducing and reducing sugar increased up until the third week of low temperature treatments after which they decreased. C/N and total sugar/N ratios were at a maximum after two weeks of low temperature treatments. Starch contents tended to decrease. After the plants were harvested for observation on January 5, 1981, the soluble nitrogen and sugar contents tended to be less than the levels during vernalization. The non-reducing sugar, total sugar and total carbohydrate contents were highest in the plants which had been exposed for three weeks of low temperatures (IIV21). Reducing sugar, non-reducing sugar and total sugar contents decreased when plants began to bolt after four weeks of low temperature treatment. Soluble nitrogen contents were higher the longer the low temperature treatments. Total nitrogen contents of all temperature treatments were lower than that of the check plot except the four-week treatment, indicating that plants began to absorb nitrogen when bolting, thus lowering both C/N and total sugar/N ratios.

Wang [19] examined that the number of tunica layers on the shoot apex of To-pe-tsai (*B. chinensis* L.) increased from 4-5 to 5-6 layers when the plant changed from the vegetative phase to the reproductive phase. This number rapidly decreased to 3 layers before the flower primordium was initiated. Radish was demonstrated the same phenomenon, with increasing sugar contents before flower bud differentiation. The sugar

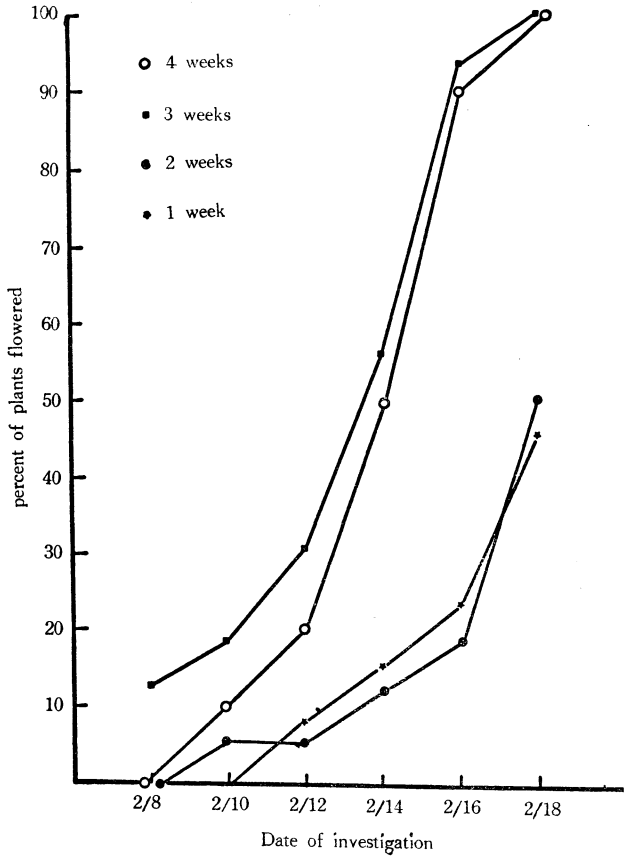


Fig. 3. Effect of different duratons of low temperature treatments (9±1°C) on the flowering behavior of Ta-Mei-Fua radish

Table 3. Changes in carbohydrate and nitrogen contents in radish root exposed to low temperatures for different duration (% dry matter)

Treatment	Nitrogen			Soluble sugar			Starch	Total carbo-hydrates	C/N	Total soluble sugar/N
	Soluble	Insoluble	Total	Reducing	Non-reducing	Total				
IV 0	1.24	1.49	2.73	21.3	14.1	35.4	7.4	42.8	15.7	13.0
IV 7	1.64	0.99	2.63	24.3	18.2	42.5	4.6	47.1	17.9	16.2
IV 14	1.39	1.17	2.56	24.4	18.7	43.1	3.8	46.9	18.3	16.8
IV 21	1.33	1.39	2.72	24.6	19.5	44.1	3.4	47.5	17.1	16.2
IV 28	1.34	1.29	2.63	23.3	17.5	40.8	2.0	42.8	16.3	15.5
IIV 0	0.98	2.14	3.12	19.5	5.9	25.4	2.2	27.6	8.8	8.1
IIV 7	1.10	1.50	2.60	17.4	9.5	26.9	4.6	31.5	12.1	10.3
IIV 14	1.18	1.71	2.89	19.9	13.2	33.1	4.8	37.9	13.1	11.4
IV 21	1.22	1.45	2.67	12.3	25.2	37.5	5.0	42.5	15.2	14.0
IV 28	1.46	1.71	3.17	13.4	14.5	27.9	6.4	34.3	10.8	8.8

content (Table 3) continued to increase as the tunica layers increased from 4-5 layers (Table 2, V14) to 5-6 layers (Table 2, IV21), and then gradually decreased (Table 2, IV28) as the tunica layers decreased to three layers. With the appearance of the flower primordium, the sugar content decreased even more (Table 3-2, IIV28), especially when plants began to bolt. This indicates that the cell division stage consumes more sugar, and that the increase and subsequent decrease in sugar contents were related not only to flower bud formation but to flower development and bolting as well.

The decrease in starch contents and increase in reducing sugar, non-reducing sugar and total sugar contents during flower bud differentiation were the same as reported by Suzuki [18] for cabbage. The results were similar also to results from previous research on the efficiency of low temperature treatments on mature kohlrabi, and showed that flower bud formation in radish increased sugar contents during vernalization. Sugar contents gradually decreased from flower primordium development to bolting. At the same time, the C/N ratio tended to decrease due to the absorption of nitrogen by the plant during bolting. Therefore, the low temperature response of flower bud differentiation was an increses in suger content, with the total sugar/N ratio reaching a peak just before flower bud differentiation. When the plants passed into the reproductive or bolting stage, sugar contents decreased and nitrogen was absorbed by the plant decreasing both the total sugar/N and C/N ratios. This pattern of metabolic changes during flower development in radish the same as demonstrated in previous research on kohlrabi (Sheen). [14]

Literature cited

1. Edward, J. T. 1966. Metabolic changes associated with vernalization of wheat. I. Carbohydrate and nitrogen patterns. Plant Physiol. 41 : 277-281.
2. Eguchi, T. and M. Koide. 1944. On the time of flower bud differentiation related to sowing-time and the effect of varnalization in *Raphnus* and *Brassica* vegetables. J. Jap. Soc. Hort. 15 (1) : 1-27. (In Japanese with English summary).
3. Eguchi, T., T. Matsumura, and T. Koyama. 1936. The effect of low temperature on flower and seed formation in Japanese radish and Chinese cabbage. Proc. Amer. Soc. Hort. Sci. 82 : 322-331.
4. Iwasaki, F. 1969. Studies on bolting in Brassica. V. Morphological and histochemical changes of the shoot apex during vernalization. J. Jap. Soc. Hort. Sci. 38 : 39-42. (In Japanese with English summary).
5. Kagawa, A. 1955. Studies on the effect of low temperature induction in radish plant. I. Studies on the transmission of thermo inducing stimulus and the effect of removing cotyledon upon the flowering induction of Japanese radish. Bull. Nat. Inst. Agr. Sci 'Jap. Ser. E4 : 265-280.
6. Kagawa, A. 1957. Studies on the effect of low temperature in radish plant. II. Res. Bull. Fac. Agr. Gifu Uni. 8 : 57-66. (In Japanese with English summary).
7. Kimura, K. 1961. Effect of temperature and nutrient on flower initiation of *Raphanus sativus* L. in total darkness. Bot. Mag. Tokyo 74 : 361-8.
8. Kraus, E. J. and H. A. Kraybill. 1918. Vegetation and reproduction with special reference to tomatoes. Oregon Agr. Coll. Exp. Sta. Bull. 149 : 1-90.

9. Kruzhilin, A. and Z. M. Shvedskaya. 1957a. Changes in sugar content during the vernalization of biennial plants. Doklady akad. mauk. S. S. S. R. 116 : 870-3.
10. Lysenko, T. D. 1935. The theoretical principles of vernalization. Agrobiology 9-64.
11. Platenius, H. 1932. Carbohydrate and nitrogen metabolism in the celery plant as related to premature seeding. Cornell Univ. Agr . Expt. Sta. Memoir 140.
12. Sadik, S. and J. L. Ozbun. 1967. Histochemical changes in the shoot tip of cauliflower during floral induction. Canadian. J. Bot. 45(7) : 955-959.
13. Sadik, S. and J. L. Ozbun. 1968. The association of carbohydrate changes in the shoot tip of cauliflower with flowering. Plant Physiol. 43 : 1696-1698.
14. Sheen. T. F. and S. C. Chuang. 1979. Effect of low temperature treatment on flower development in Peiping kohlrabi. Jou. of Agr. Res. of China 28(2) : 67-77.
15. Shinohara, S. 1950. Studies on the bolting of Japanese radish. Commemoration Issue of 50th Anniversary of Shizuoka Agr. Exp. Sta. 183-221. (In Japanese with English summary).
16. Shinohara, S. 1959. Genecological studies on the phasic development of flowering centering on the cruciferous crops, especially on the role of vernalizatoin on ripening seeds. Shizuoka prefecture agri. Exp. Sta. Japan. Technical Bulletin 6 : 1-166.
17. Sugiyama, N. 1942. Varietal difference of bolting behavior of radish and Brassica. Agri. and Hort. 17 (1) : 1453-1455. (In Japanese)
18. Suzuki, Y. 1972. Studies on vernalization in some cole and root vegetable, especially on the transition of low temperature sensitivity and the metabolic change during vernalization. Fac. Agr. Tokyo Uni. of Eduction 18 : 27-92 (In Japanese with English summary).
19. Wang, P. J. 1969a. Changes in the anatomical structure of the shoot apex of To-pe-tsai (*Brassica chinensis* L.) during transition form the vegetative to the reproduciive state and the determination of the bolting. J. Jap. Soc. Hort. Sci. 38 : 52-9.
20. Yamato K. 1932. Effect of low temperature on flower promotion. Agri. and Hort. Vol. 7 (1) : 861-868. (In Japanese).
21. Yamasaki, K. 1962. On the flower formation in some vegetable crops, with special reference to the transitional susceptibility of the growing towards the environmental factors responsible for the flower differentiation. Bull. Hort. Res. Sta. Japan. ser. B. No. 1 : 88-141. (In Japanese with English summary)

大梅花蘿蔔低溫處理與開花之關係[1]

沈 再 發[2]

摘 要

本試驗以種子春化型之蘿蔔爲材料，一方面研究亞熱帶地區選種育種上對於較晚抽性母株之開花促進方法，一方面研究其低溫處理時，莖頂（Shoot apex）的發育過程與組織內氮和碳水化物之代謝情形。

經試驗結果大梅花蘿蔔成株，於選拔後剪去葉身，以根行 9±1°C 之有光低溫處理三週，可達完全抽苔開花。

以成株行低溫處理二週時，其莖頂之外鞘層（Tunica layer）由營養生長之 5 層變爲 6 層，低溫處理三週時又轉變爲 3 層，同時莖頂之細胞分裂旺盛，內體層（Corpus layer）之細胞形狀由平坦狀轉變爲豐滿之豎立狀。經四週低溫處理開始形成花原基。在低溫處理期間糖類增加，其含量達最高時，莖頂之細胞分裂旺盛，外鞘層轉爲 5 — 6 層。當外鞘層減爲 3 層和花原基形成時，糖類隨而下降。此種糖類之消長狀況與綠植物春化型之成株低溫處理之變化大致相同。

---

1. 臺灣省農業試驗所 研究報告 第 1015 號。
2. 本所研究員兼鳳山熱帶園藝試驗分所分所長。臺灣省 高雄縣 鳳山市。