THE EFFECT OF FROST TREATMENT ON TURNIP RAPE SEEDLINGS, RIPENING SEEDS AND THEIR FATTY ACID COMPOSITION

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INTRODUCTION

Oilseed crops, spring turnip rape (*Brassica campestris* L.) and spring rape (*B. napus* L.), are sown in Finland during the first two weeks of May. In the main turnip rape growing area, which is in the southern and central parts of the country, the minimum ground temperature may fall below 0°C during 10-15 nights in May. Nightfrost occurs even in June (Heino and Hellsten 1980). On occasions the frost has been rather severe (-5°- -6°C) causing serious damage to cotyledonous seedlings in oilseed fields. During seed ripening in August local nightfrost is also common. Frost drastically reduces the seed yield, and losses up to 50% have been described in *B. juncea*, for example (Elf and Ohlsson 1987).

Breeding for improved fatty acid composition in zero erucic acid rapeseed varieties in Finland is directed at increasing the linoleic acid content (Hovinen and Laakso 1987). On the other hand, rapeseed oils with a clearly lower level of technically undesirable α-linolenic acid (n-3) are needed by the margarine industry. Reducing n-3 fatty acid levels may be problematic, since hexadecatrienoic acid, which is indicative of leaf damage (Vance and Stumpf 1978), is also n-3 fatty acid.

The aim of this study was to examine the frost sensitivity of spring turnip rape seedlings in cotyledenous stage and seeds in podfilling stage. Two standard varieties commonly cultivated in Finland, and a breeding line with a low α-linolenic acid content were used in the controlled experiments. Changes in the fatty acid composition of the frost-treated seedlings and developing seeds were also studied.

MATERIALS AND METHODS

Two standard varieties of spring turnip rape (Emma WW, type 0, Kova Sv, type 00) and a low α-linolenic acid line (Hja 91285-25, type 000) were used. Seeds were sown in a moistened mixture of sand and peat (1:2) in 10x10cm boxes, 25 seeds in each, covered with a layer of sand (2cm).

Frost Treatment of Seedlings

The seedlings were grown in two climate chambers (day 16°C or day 16°C/night 5°C). The daylength was 16 hours. Eight days after sowing the cotyledonous plants were subjected to the frost treatment. Three seedling boxes of each variety were placed in the chamber, and the temperature lowered to -1°, -3° or -5C° wihin one hour. The frost treatment (3h) was applied at the end of the dark period, after which the plants were kept at the temperatures listed above. Damage was recorded at 2, 26, 56 and 144 hours (6 days) after treatment. Six days after treatment the surviving seedlings were cut and the height, dry weight and leaf area were measured. Damaged and destroyed plants were counted.

Frost Treatment During Seed Ripening

The same seed material was grown in a mixture of sand and peat (1:2) in containers (7 l), eight plants in each. The containers were kept in a greenhouse (day at 22°C, night at 15°C) until the beginning of flowering, when they were moved to a flowering turnip rape field. At the end of flowering the containers were placed in the climate chambers at 16° (a) or day 16° and night 5°C (b). Five days later the plants in chambers a and b were subjected to the frost treatment at -2° for 3 hours. The ripened plants were harvested four weeks later.

The fatty acid composition of the dried seedlings and seeds from ripened plants was analysed by GC (Laakso 1986). The composition of the original seed materials are presented in Table 1.

Table 1. Fatty acid composition of the seeds of standard varieties and a low α-linolenic acid (18:3) breeding line used in the experiments.

Fatty acids (%)	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
Emma	3.4	0.2	1.3	57.7	22.8	12.0	0.4	1.3	0.2	0.7
Kova	3.2	0.2	1.3	56.2	24.9	12.6	0.4	1.0	0.2	0.2
Hja line	3.3	0.2	1.2	59.7	26.1	8.0	0.4	0.9	0.2	-

Statistical differences were studied by analysis of variance and t-test.

RESULTS AND DISCUSSION

Effect of Frost Treatment on Seedlings

Growing temperature had a clear effect on the growth (Table 2). The height and leaf area of the hardened seedlings grown at 16°/5°C were smaller than those grown at 16°C. The reduction in height of the survived seedlings, measured 6 days after the treatment at -5° was also significant. The treatment only slightly affected on the growth of the variety 'Emma'.

Table 2. The effect of frost treatment (-5°C) on mean height, leaf area and dry weight of the seedlings 6 days after treatment.

Material	Emma		Kova		Hja 91285-25	
Growing temp.	16°	16/5°	16°	16/5°	16°	16/5°
Height, cm						
no frost	2.5	1.3	2.1	1.1	3.3	1.6
-5°	1.8	1.0↓↓	1.6↓	0.8↓↓	2.5↓↓	1.4
Leaf area, cm^2						
no frost	190	136	177	118	177	111
-5°	84↓	124	131	78↓	105↓	72↓↓
Weight[1], g						
no frost	0.6	0.6	0.5	0.5	0.6	0.5
-5°	0.3	0.5	0.4	0.3↓	0.3↓	0.3↓↓

[1]Mean of 10 plants; unpaired t-test: ↓↓$p<0.01$; ↓$p<0.05$

Significant differences were found in frost tolerance between the materials. In unhardened seedlings, grown at 16°C (Fig. 1), the frost treatment at -3°C caused obvious damage; the differences were significant between the 'Kova' and Hja line. However, serious injury at -5° was found in 35-67% of the seedlings.

In the hardened seedlings, grown at 16°/5°C, damage was found two hours after treatment at -5°, however, the damage later on became clearly worse. The seedlings were tolerant of frost at -3°C without practically any damage, whereas the treatment at -5° gave different results. The variety 'Emma' clearly had better frost tolerance (18% damaged) compared with the other materials. There was no significant difference in frost injury between 'Kova' and a low α-linolenic acid breeding line. These results suggest that considerable differences in hardening ability of spring turnip rape varieties can be found.

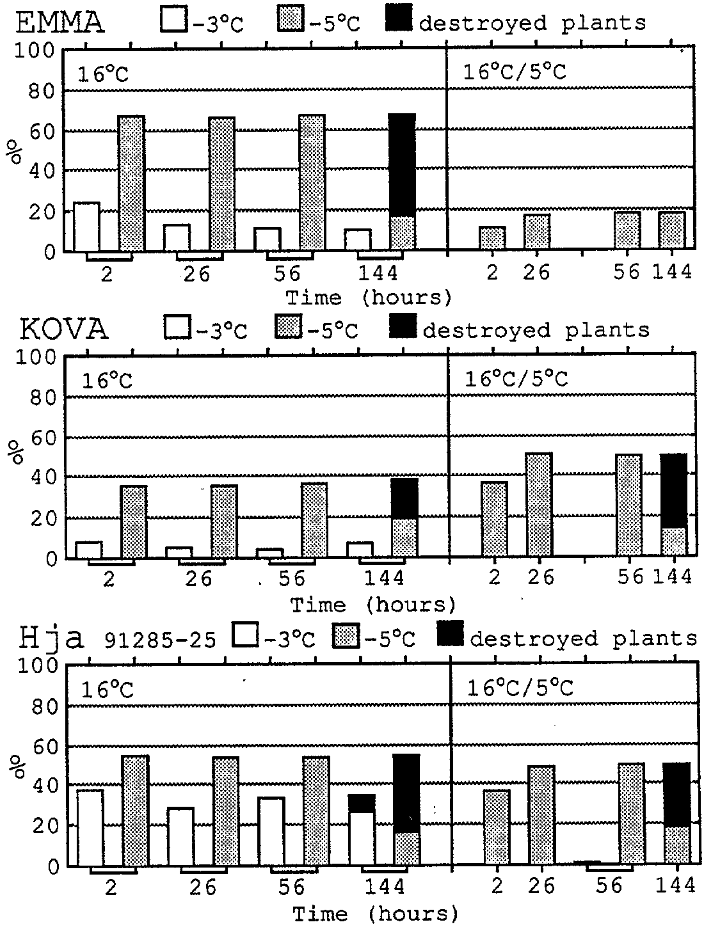

Fig. 1. Percentage of damaged (▪) and destroyed seedlings (■) after frost treatment at -3° and -5°C. Plants grown in chambers (day 16° or day 16°/night 5°C), observations made at 2, 26, 56 and 144 hours.

Effect of Frost Treatment on Fatty Acid Composition

Changes in the relative fatty acid composition of the hardened seedlings were very similar, especially in the case of n-3 fatty acids (Table 3). The proportion of hexadecatrienoic acid (16:3) increased at -5°C compared to the untreated plants. The changes were significant in all the materials. However, the treatment had no effect on the levels of the principal component α-linolenic acid (18:3) (Table 3).

Table 3. Changes at hexadecatrienoic (16:3) and α-linolenic acid (18:3) levels in hardened seedlings (grown at 16/5°) after treatment at -5°C.

Material	EMMA		KOVA		Hja-91285-25	
Fatty acid (%)	16:3	18:3	16:3	18:3	16:3	18:3
no frost	9.8	55.2	10.0	55.2	9.6	54.5
-5°C	11.4↑	56.1	12.8↑	56.1	12.8↑	55.4

unpaired t-test: ↑$p<0.05$

Table 4. Changes at oleic (18:1), linoleic (18:2) and α-linolenic acid (18:3) levels in ripening seeds of unhardened (16°C) and hardened plants (16/5°) after treatment at -2°C.

Material	EMMA			KOVA			Hja-91285-25		
Fatty acid (%)	18:1	18:2	18:3	18:1	18:2	18:3	18:1	18:2	18:3
Unhardened	55.8	22.9	14.1	55.6	22.6	14.5	55.6	25.2	11.5
Hardened	58.3↑	22.7	11.9↓↓	58.6↑	23.0	11.2↓↓	57.9	24.8	9.9↓

unpaired t-test: ↑,↓ $p<0.05$; ↓↓ $p<0.01$

In ripening seeds, the proportion of α-linolenic acid decreased significantly (Table 4), but no varietal differences were found. The level of oleic acid (18:1) increased ($p<0.05$) in both varieties ('Emma', 'Kova') whereas the proportion of linoleic acid remained unchanged in all the materials. The results of the fatty acid analyses show that the changes at the n-3 fatty acid levels caused by frost are similar in low (≈8%) and high (≈12%) α-linolenic acid seed materials.

CONCLUSIONS

Hardened seedlings of spring turnip rape varieties tolerate frost rather well at -3°C, whereas frost at -5° causes serious injury which can later on even become worse. Varieties have clearly different hardening ability at the cotyledonous stage. The results also suggest that the α-linolenic acid content, which is a few percent units lower than normal (≈12%), does not affect the frost tolerance of the seedlings. The testing of frost tolerance and hardening ability is especially important when breeding varieties to be cultivated in northern marginal areas.

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