Character Expression of Induced Dwarf Mutants in Rice

II. Morphological and Histological Observations on the Effects of Temperature on Culm Elongation in the Dwarf Mutant, Fukei No. 71.

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Following previous studies (KITANO and FUTSUHARA 1981), the effects of temperature on culm elongation of the dwarf mutant, Fukei No.71 and its original cultivar, Fujiminori were evaluated in the present investigation under eight different temperature conditions composed of the combination of two temperatures, high (30°C–25°C) and low (23°C–18°C), and three growth periods (vegetative, early reproductive, and late reproductive or culm elongation stage). Fukei No.71 showed a larger response to the treatments in culm elongation than Fujiminori, the culm elongation of the former being reduced significantly by high temperature treatment in the culm elongation stage. On the other hand, under low temperature conditions in this stage, it grew as tall as Fujiminori. To elucidate the dwarfing mechanism of Fukei No.71, some histological observations were carried out on the elongated internodes of Fukei No.71 and Fujiminori grown under high and low temperature conditions. When Fukei No.71 was grown under high temperature, it showed a tendency for a decrease in the number of parenchyma cells in the longitudinal direction and an increase in number in the transverse direction of each elongated internode. It was also observed that partially non-uniform parenchyma cells occurred only in Fukei No.71 when treated with high temperature. These results suggest that the dwarfness expressed under high temperature condition in Fukei No.71 is induced by abnormal cell differentiation from intercalary meristems of the internodes.

Introduction

The character expression of mutant genes is under the regulations of some environmental factors (YAGIL and STEBBINS 1969, WESTERMAN and LAWRENCE 1970, WESTERMAN 1971, GUSTAFSSON and DORMLING 1972). Genetic dwarfness is one of the most inducible and recognizable mutations. Some studies have been carried out on the interaction between genetical and environmental effects (GORTER 1961, POLLOCK 1962, TAKAHASHI 1974, CANVIN and MCVETY 1976).

In the previous work (KITANO and FUTSUHARA 1981), it was shown that the rice dwarf mutant, Fukei No.71 expressed the reduction of culm elongation to a greater extent than its original cultivar, Fujiminori under high temperature conditions.

The present experiment was undertaken to make clear the morphological bases for the interaction between gene action and the effect of temperature on culm elongation, and to elucidate the mechanism of the dwarfing of Fukei No.71 from the histological viewpoint.

Materials and Methods

Temperature treatment : Fukei No.71 and Fujiminori seedlings were grown until

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Fig. 1. Diagram explaining the eight temperature conditions employed in the experimental treatments.

Fig. 2. Schematic illustration of longitudinal section indicating the measured cell layers of parenchyma of internode.

the 2.5th leaf-age stage in the greenhouse with soil-pot culture, at a rate of four plants per each 1/5000 are-wagner pot. Three replicated pots (12 plants) were tested under each of eight different temperature treatments composed of the combination of two temperature conditions and three growth periods (Fig. 1). Two temperature treatments, high (day 30°C-night 25°C) and low (23°C-18°C), were performed using the phytotron of Faculty of Agriculture, Nagoya University. The growing period was divided into three stages according to the leaf-age as shown in Fig. 1. The time changing temperature conditions was decided with the anatomical observations in preliminary experiment. From these observations, it was established that the initial reproductive phase (panicle initiation) of two lines was induced at the 8th and 7th leaf-age stages, respectively, when these two lines were grown under high (30°C-25°C) and low (23°C-18°C) temperature conditions throughout the vegetative growth period. Therefore, the first conversion of the temperature condition was carried out at these leaf-age stages. The second conversion was done at the (n-1) leaf stage (n refers to final leaf number developed on the main culm), at which the primary phase of culm elongation could not yet be found.

Morphological and histological observations of culm elongation: In each of two lines, twelve plants were grown until mature stage under high or low temperature conditions throughout the whole growth period. After measuring some characters of the shoots, longitudinal and transverse thin sections of each elongated internode were observed microscopically. Paraffin and JB-4 plastic embedding methods were used for making these sections. Cell length and width were measured for cells of the second or third layer from the external and internal sides of parenchyma, respectively (Fig. 2). The longitudinal cell number was calculated as follows: internode length/mean cell length for the three

levels of parenchyma, using longitudinal sections of upper, middle and lower parts of each internode.

Results

Culm and panicle elongation : A diagram of culm elongation pattern and mean values of some characters in two lines grown under the different temperature conditions are shown in Fig.3 and Table 1, respectively. Fukei No.71 had larger response of culm elongation to the treatments than Fujiminori ; its coefficient of variation for the treatments ($\frac{\sigma_{n-1}}{\bar{x}} \times 100$) was 23.88, compared with 6.33 for Fujiminori. The culm elongation of Fukei No.71 was remarkably reduced when the culm elongation period passed through high temperature condition (T_1 , T_2 , T_3 and

Fig. 3. Comparison of culm elongation patterns between Fujiminori and Fukei No.71 grown under eight different temperature conditions.

Table 1. Mean values of some plant characters in Fujiminori (F) and Fukei No.71 (71) grown under eight different temperature conditions

Character	Line	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈
Culm length (cm)	F	77.6	68.7	79.6	69.8	71.0	75.3	73.1	69.7
	71	37.6***	34.3***	42.2***	39.0***	47.4**	62.0	52.5***	65.0
1st internode length (cm)	F	37.6	31.2	38.2	33.0	33.4	30.8	33.1	29.8
	71	22.8***	22.4***	26.0**	24.5**	26.1**	25.5**	27.5***	27.8*
2nd internode length (cm)	F	18.9	18.5	22.5	21.3	20.5	21.3	23.0	22.3
	71	7.8***	4.8***	11.5**	10.2***	16.0	19.7	16.0	20.0
3rd internode length (cm)	F	11.7	13.0	12.9	10.5	9.9	12.3	11.7	12.2
	71	4.9**	3.7***	3.1***	2.8***	3.8**	13.2	7.9*	12.4
4th internode length (cm)	F	6.7	5.7	6.1	4.9	6.6	7.7	5.3	5.7
	71	1.9*	3.3	1.4***	1.5**	1.5**	2.8*	2.1***	4.8
Panicle length (cm)	F	21.3	19.6	19.9	18.3	19.7	18.1	19.9	17.6
	71	20.0	17.8*	19.5	18.6	18.6	16.9	18.8	16.7
Final plant height (cm)	F	102.5	90.8	107.1	92.6	98.1	95.3	94.4	89.0
	71	67.6***	65.9***	74.9***	69.4**	69.8***	78.3*	74.8***	85.4
Panicle number	F	2.5	3.3	3.0	3.0	2.9	3.3	3.6	2.8
	71	4.3**	4.3**	3.5	3.2	4.6*	4.2**	4.5	3.4*
Duration to heading (days)	F	58.9	63.3	68.2	72.9	65.7	76.5	68.3	84.7
	71	58.8	61.8	67.5	74.4	64.8	77.6	67.3	85.7
Final leaf number	F	11.8	11.6	11.0	11.0	11.6	11.3	11.5	11.0
	71	12.0	11.5	11.3	11.2	11.8	11.3	11.5	11.0

*, ** and *** : Significantly different from the original line at the 5, 1 and 0.1% levels, respectively.

Table 2. Analyses of variance for culm and panicle lengths of Fujiminori and Fukei No.71 responding to temperature treatments

	Culm Length				Panicle Length			
	D. F.	S. S.	M. S.	F	D. F.	S. S.	M. S.	F
Replication	2	20.92	10.46	1.32	2	0.08	0.04	0.10
Line (L)	1	7872.00	7872.00	996.22***	1	10.45	10.45	24.58***
Temperature (T)	7	1491.34	213.05	26.96***	7	54.58	7.80	18.34***
L × T	7	1605.57	229.37	29.03***	7	4.33	0.62	1.45
Error	30	237.06	7.90		30	12.75	0.42	

*** : Significant at the 0.1% level.

Table 3. Analyses of variance for culm length of Fujiminori and Fukei No.71 responding to temperature treatments

Factor	Line	(Fujiminori)				(Fukei No.71)			
		D. F.	S. S.	M. S.	F	D. F.	S. S.	M. S.	F
Replication		2	17.54	8.77	1.26	2	37.35	18.68	2.47
Temperature effect		7	343.56	49.08	7.07**	7	2753.36	393.34	51.98***
Temperature effect in the 3rd period		1	15.68	15.68	2.26	1	2047.95	2047.95	270.63***
Pretreatment effect ⁽¹⁾ (- - H)		3	273.10	91.03	13.12***	3	97.50	32.50	4.29*
Pretreatment effect ⁽²⁾ (- - L)		3	54.78	18.26	2.63	3	607.91	202.64	26.78***
Error		14	97.14	6.94		14	105.94	7.57	

Note. (1) : Temperature effect before high temperature treatment in the third period.

(2) : Temperature effect before low temperature treatment in the third period.

, ** and *** : Significant at the 5, 1 and 0.1% levels, respectively.

T₄ in Fig.3). The varietal difference in temperature response between Fukei No.71 and Fujiminori was examined statistically, using the method of variance analysis with three replications (Table 2). Significant main effects (line and temperature) were detected for both culm and panicle length. In addition to these effects, a significant interaction of line×temperature was found in culm length. Therefore, the effect of temperature in three growth periods was analyzed for culm length in each Fukei No.71 and Fujiminori, separately. Then, sum of squares (s.s.) of temperature effects (d.f.=7) in each line was broken down into s.s. of the temperature effect in the third period (d.f.=1) and s.s. of temperature effects before each of the high (d.f.=3) or low (d.f.=3) temperature treatments in the third period. These results are given in Table 3. Fukei No.71 presented an excessively large F value for the temperature effect in the third period, although that of Fujiminori was not significant. An opposite tendency was found in the temperature treatment effects of first and second growth periods between these lines.

Some other characters : As shown in Table 1, duration to heading was distributed in a range extending from 59 days (T₁) to 86 days (T₈). Final leaf numbers developed on the main stem of both lines were 11 or 12, which seemed to be affected by the temperature condition that prevailed during the vegetative growth period. No significant differences were found in these two characters between two lines on each treatment. Fukei No.71

Fig. 4. Typical examples of mature plants (A), naked culm (B) and some internodes (C) of Fujiminori and Fukei No.71 grown under high and low temperature conditions.

Note. 1. (H) : (30°C-25°C) (L) : (23°C-18°C) 2. F : Fujiminori 71 : Fukei No.71
3. Each group of internode (In.) in plate C showing In. 2 (left), In. 3 (center) and In. 4 (right).

developed more panicles than Fujiminori in some treatments.

Histological observations of culm elongation : The mature plants and some elongated internodes for each of the two lines grown under high or low temperature conditions throughout the whole growth period are illustrated in Fig.4. The mean values for some morphological and histological characters of elongated internodes are given in Table 4. When Fukei No.71 was grown under high temperature conditions, it had a slender culm diameter with a thick culm wall. There were significant differences between these two lines in the both longitudinally and transversely cell numbers for all internodes under the high temperature conditions. In addition, a remarkable varietal difference was found in the parenchyma cell form of the internode as shown in Fig.5. In transverse sections of internodes of Fukei No.71 treated with high temperature, many small parenchyma cells which had narrow cell widths were observed (Fig.5-B). Some excessively long or short cells were observed in longitudinal sections (Fig.5-F). Such abnormal cell forms were also found in the other internodes of Fukei No.71 grown under high temperature condition. However, this characteristics for Fukei No.71 were not apparent under the low temperature conditions, and the cell form was similar to that of Fujiminori (Fig.5-C, D and G, H). There were no significant differences between these two lines under any temperature conditions for the other characters, such as number of inner and outer vascular bundles and number of xylem, phloem and sclerenchyma cells of each internode.

Fig. 5. Transverse and longitudinal sections (5 μm) of internode (In. 3) of Fujiminori and Fukei No. 71 grown under high and low temperature conditions.

Note : Plate A-D, transverse section.

E-H, longitudinal section.

A and E, Fujiminori (30°C-25°C)

B and F, Fukei No. 71 (30°C-25°C)

C and G, Fujiminori (23°C-18°C)

D and H, Fukei No. 71 (23°C-18°C)

—=100 μm

Table 4. Comparison of morphological and histological characters of internodes between Fujiminori (F) and Fukei No.71 (71) grown under high and low temperature conditions

Character	Line	High temperature (30°C–25°C)				Low temperature (23°C–18°C)			
		Internode				Internode			
		In. 1	In. 2	In. 3	In. 4	In. 1	In. 2	In. 3	In. 4
Internode length (cm)	F	38.3	20.7	14.4	9.4	30.7	20.7	14.4	5.4
	71	27.5***	9.8***	5.5***	3.1***	28.4	16.0	10.3	5.4
Outer diameter (10 ⁻¹ cm)	F	1.81	3.28	3.71	4.05	1.62	3.31	4.03	4.60
	71	1.56*	2.31***	2.75**	3.11***	1.69	3.11	3.93	4.67
Thickness of culm width (10 ⁻² cm)	F	3.47	3.85	3.77	5.47	3.52	4.48	4.68	6.81
	71	4.21	5.88**	6.35**	7.24*	3.93	4.71	5.38*	7.22
Transverse cell width (μm)	F	31.5	29.6	30.5	33.4	26.2	35.9	32.8	33.8
	71	22.9*	22.9*	27.2	26.7*	28.6	33.1	32.8	30.8
Longitudinal cell length (μm)	F	84.1	107.6	100.8	108.7	87.3	113.5	94.5	98.8
	71	121.7***	112.3	86.0	108.3	102.4**	104.6	98.1	109.1
Transverse cell number	F	10.8	11.2	12.4	15.4	11.2	11.2	13.6	18.0
	71	15.2*	21.4***	20.3***	20.3**	11.2	12.4	15.4*	19.2
Longitudinal cell number	F	4647	1980	1638	1024	3560	1955	1529	682
	71	2525*	1041**	793**	326***	3300	1705	1098***	563
No. of inner vascular bundles	F	9.6	26.2	27.8	27.6	9.8	28.6	27.4	31.0
	71	8.6	25.6	27.4	28.0	9.8	26.8	26.0	29.8
No. of outer vascular bundles	F	16.8	23.4	24.6	24.8	16.0	24.2	24.6	25.2
	71	13.8	22.4	24.8	24.0	16.2	23.0	23.8	24.8

*, ** and *** : Significantly different from the original line at the 5, 1 and 0.1% levels, respectively.

Discussion

In general, dwarf mutants are classified into two types. The dwarf gene may cause a reduction either in the plant size as a whole (nanism), or it may cause a decrease in size of only one or some specific organs in plant bodies (brachysm) (Cook 1915). The induction of both of these two type of mutants has been reported in rice plants. The two dwarf mutants induced from Hosetsu (SHINBASHI *et al.* 1974) and Akibare (FUTSUHARA 1974) are typical examples of dwarf mutants of the former type, while some mutants of "dm" type (TAKAHASHI and TAKEDA 1969, YAMAGUCHI 1974) are examples of latter type. The phenotype of Fukei No.71, where a reduction in culm elongation is expressed under high temperature conditions (KITANO and FUTSUHARA 1981), is thought to be of a "brachysm" type, although its character expression varies widely according to the growth temperature conditions.

MAHON and CANVIN (1972) reported that so called Type 1 dwarf, which was selected from the hybrids of Kenya Farmer × Marquillo in wheat, expressed the dwarfness character only when a low temperature (16°C) treatment was employed for 12 hours at seedling stages, and this dwarfness was due to the lack of cell division caused by the low temperature. The present results showed that high temperature treatment given at late reproductive stage had most intense effect in reducing culm elongation in Fukei No.71.

The effects of treatment before the third period (late reproductive stage) were also significant (Table 3). These results suggest that the character expression of the dwarf gene in Fukei No.71 is affected not only by temperature conditions during the culm elongation period, but also by temperature conditions before the third period. Further detailed investigations are needed to elucidate whether temperature conditions at various growth stages affect the reduction of culm elongation of Fukei No.71.

On the other hand, several studies have been reported on the cytohistological basis of dwarfism in gramineous crops; they include those on wheat (CHAWDHRY and ALLAN 1966), maize (ABBE and PHINNEY 1942, HANSEN 1957) and rice (KAMIJIMA 1981). PELTON (1964) described that fewer cell divisions, less cell elongation, a change in rate of differentiation, or a combination of two or more of these at cellular level led to the dwarfism at the organ level. The present results show that the dwarfness of Fukei No.71 grown under high temperature conditions is caused mainly by a decrease in cell number in the longitudinal direction of the culm (Table 4). On the other hand, Fukei No.71 possessed a larger number of cells in the transverse direction than Fujiminori under the high temperature conditions. These changes in cell number may be caused either by less cell destruction in the inner parenchyma or by an increase in number of cell division in the transverse direction in the intercalary meristem in Fukei No. 71, as compared with that of Fujiminori. Furthermore, abnormal cell forms (excessively long or short cells) were observed in some parenchyma regions only in Fukei No.71 under high temperature conditions (Fig.5-F). These parenchyma cells of elongated internodes originate from the intercalary meristem; they differentiate at culm elongation stage in rice plant (KAUFMAN 1959, KAWAHARA *et al.* 1968). From these observation and other considerations, it is suggested that the dwarfness of Fukei No.71 in response to high temperature treatment may due to abnormal cell differentiation (cell division and/or cell elongation) in the intercalary meristem. Further detailed developmental analyses of this problem will be dealt with in future studies.

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イネにおける矮性突然変異の形質発現

II. 矮性突然変異系統ふ系71号における節間伸長の温度反応と 伸長節間の組織形態学的観察

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前報において矮性突然変異系統ふ系71号の節間伸長は、原品種および他の突然変異系統とは著しく異なった温度反応を示すことを明らかにした。本報ではふ系71号の節間伸長におよぼす生育温度の影響をさらに詳細に調べるとともに、異なった温度条件下で伸長した各節間の外部および内部形態的特徴を明らかにする目的で行った実験結果について報告する。

突然変異系統ふ系71号および原品種フジミノリの生育期間を3段階(栄養生長期, 生殖生長前期, 生殖生長後期)に区分し, ファイトトロンを用いて高温(30°C~25°C)および低温(23°C~18°C)下で両系統をポット

栽培した。処理温度と処理期間の組み合わせで合計8種類の異なる処理を行った結果、ふ系71号の稈長は、本実験で用いた処理範囲内で、フジミノリに比較して大きな変動を示した。さらにふ系71号は、生殖生長後期、すなわち節間伸長期にあたりと考えられる時期の高温に最も強く反応して短稈化を示した。このような傾向はフジミノリでは全く認められず、ふ系71号の特異的な温度反応であることが確かめられた。

次に高温および低温下で生育させた両系統の各伸長節間における縦断および横断切片を作成し顕微鏡観察を行ったところ、ふ系71号では、高温処理によってフジミノリに比較して各節間における柔組織の縦列細胞数が減少し、逆に横列細胞数は明らかに増加する傾向が認められた。さらに、ふ系71号の各節間を構成する柔細胞(parenchyma cell)は、高温処理によって不斉な異常形態を示すことも観察された。これに対し、低温処理区におけるふ系71号の各節間組織とフジミノリのそれとの間には明確な形態的差異は認められなかった。

以上の結果より、ふ系71号の矮性遺伝子は、その発現にさいし、生育温度、とくに節間伸長期の生育温度の影響を強くうけ、この時期の高温によって大きく短稈化作用を示すことが明らかとなった。また各節間の形態観察の結果から、この矮性遺伝子は、伸長節間を構成する細胞の分化、すなわち節間分裂組織の形態形成に関与しているものと推定された。

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