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Inheritance and chromosomal location of flecking in a mutant C591(M8) of wheat (*Triticum aestivum* L.)

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Abstract

Genetic analysis of data in F_2 generation derived from the cross NP852/C591(M8) and NP846/C591(M8) revealed that flecking in mutant C591(M8) of *Triticum aestivum* L. is controlled by a single dominant gene. The mutant C591(M8) was also crossed with individual monosomic lines of Chinese Spring and monosomic F_1 plants were cytologically identified. The F_2 plants derived from individual monosomic F_1s were scored for the presence of flecking. No critical line could be identified as several lines deviated from expected Mendelian ratio. Considering the peculiar characteristics of the mutation, which resembled the disease lesion mimic mutations reported in other crops such as maize, this indeed is a mutation in hexaploid wheat. The gene symbol *Flk* is proposed for flecking (= lesion mimic) in the mutant line C591(M8). The flecking mutant will serve as a useful genetic marker.

Key words: Bread wheat, flecking, disease lesion mimic mutant, inheritance, chromosomal location

Introduction

Genetic analysis in any species is primarily intended to understand the nature and mode of inheritance of different characters. Genetic markers are used in preparing linkage maps which are valuable tools for basic studies and manipulating the plants for improvement with respect to various traits.

A large number of mutant traits are reported among which chlorophyll deficient mutant(s) are analysed in different crops such as maize, wheat, *Pennisetum* spp., rice, soybean, sunflower, pea, *Arabidopsis* and sweet clover etc. In wheat, chlorophyll deficient mutants have also been reported and their inheritance were studied as early as in 1933 [1]. Many aneuploid plants that lack either a complete group of 5 chromosome or the long arm of a group 5 chromosome exhibit a characteristic orange-to-brown flecking of young leaves when grown under cool short days [2]. Flecked mutants were recorded but tests have not been carried out to see if these carry alterations to group 5 chromosomes. Later, a number of chlorophyll or disease lesion mimic mutants were reported and their mode of inheritance, physiological and ultrastructural aspects were studied [3-6]. Recently attention is being paid to another class of mutants called disease lesion mimics that display a phenotype resembling symptoms caused by pathogen attack particularly in maize [7, 8]. Interestingly, disease lesion mimic mutants often exhibit induction of defense responses which are typically upregulated at the time of pathogen infection [7, 9] that represent a valuable resource for studying the intricacies of plant defence mechanism and the ubiquitous association of these mimics with cell death. These mutants generally show symptoms like chlorosis or necrosis even in the absence of any pathogen. The present investigation deals with the phenotypic expression of flecking trait, its mode of inheritance and attempts to locate them on specific chromosome.

Materials and methods

The materials consisted of bread wheat (*Triticum aestivum* L.) cultivars, namely, NP846 and NP852 and flecking mutant C591(M8) and monosomic series of Chinese Spring(CS). Mutant C591(M8) was obtained by treatment of C591 seeds with Nitrosomethyl urethane (0.03%) by Kumar [10]. The mutant plants display characteristic yellowish spots on leaves and leaf sheath from the boot leaf stage onward, while NP846 and NP852 produced normal green leaves and leaf sheath. C591(M8) was crossed with NP846, NP852 and chromosomally identified individual monosomic plants

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of Chinese Spring. The F_1 NP846/C591(M8), NP852/C591(M8) and 20 monosomic/C591(M8) were grown during *rabi* 2003-04. The F_2 generation obtained from normal crosses and derived from cytologically identified F_1 monosomics were raised during 2004-05 under normal field conditions. Normally the presence of flecks (lesion mimics) on mutant C591(M8) are detectable at early stage. Characteristically yellow spot starts appearing at boot leaf stage. The development of yellowish spots begin randomly from lowest leaf sheath and leaf and progresses towards the top as the plant grow (Fig. 1) and spotting and intensity is variable. The observations on presence and absence of flecking were carefully recorded and plants were classified into two categories. The data were subjected to the Chi-square test for testing the goodness of fit. The counting of chromosome number in monosomic series and the monosomic F_1s was done at meiotic metaphase I as per standard procedure.

For light microscopy, cross sections of the leaf sheath of both normal and fully flecked mutant plants were taken and mounted in 20% glycerine solution and observed under Olympus PM 10 ADS photomicrographic system. Leaf sheath from normal and mutant plants were fixed in ethanol/chloroform (3:1) mixture containing 0.15% trichloroacetic acid and cross sections were stained using 0.01% Aniline blue. The stained material was observed by Nikon Microphot-FX microscope with fluorescence attachment, illuminated with 200 W high pressure mercury lamp. The observations were taken with B (380-490 nm) and /or BG 38 (650nm) excitation filters in combination with BA 520 or BA530 barrier filters.

Results and discussion

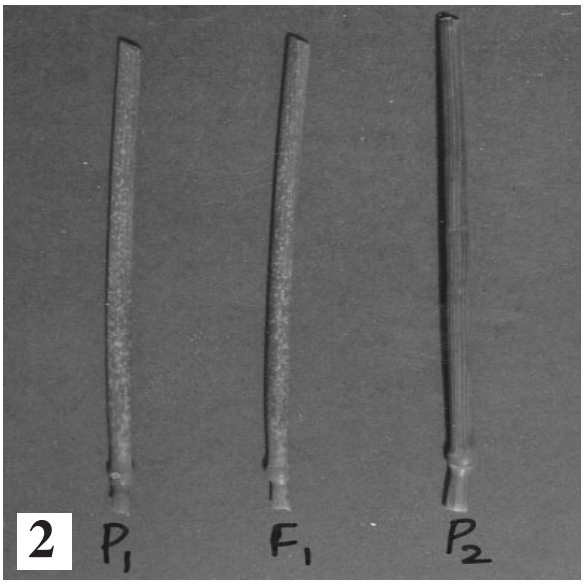
The leaves and leaf sheath of F_1 plants produced flecks (mutant phenotype) in both NP846/C591(M8) and NP852/C591(M8) crosses (Fig. 2). The F_2 generation segregated into two phenotypic classes and the data fit well in a 3 flecked : 1 normal (non-flecked) ratio with non-significant χ^2 value (Table 1), indicating that flecking trait is controlled by a single dominant gene. The flecking mutant C591(M8) which shows characteristic yellowish spots on the leaves and leaf sheath was induced through chemical mutagenesis. Flecking mutant was one of the 11 mutants originally isolated for leaf rust resistance but the peculiar chlorophyll deficient mottling of leaf surface (flecking) of this mutation was not reported [8]. However, its stability and mode of inheritance was investigated by Sudha and Tomar [11] under rust free condition. They also studied in detail the leaf anatomical features and

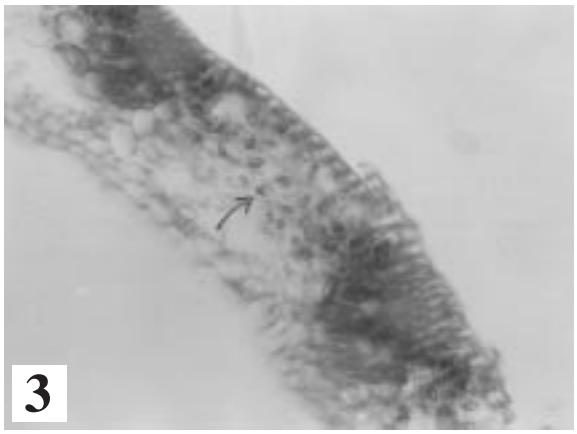
Table 1. Segregation for flecking trait in F_2 generation

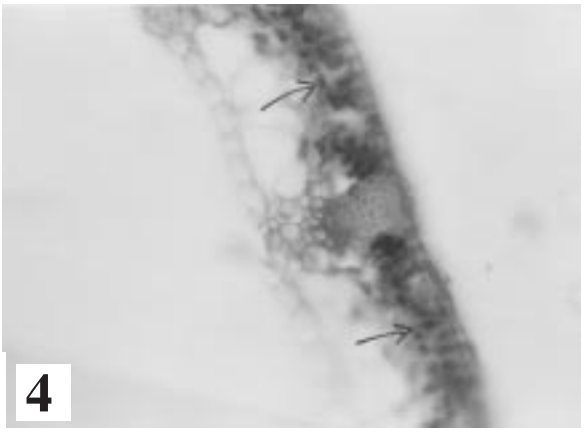
Parents/ cross	Gene-ration	No. of plants		Total	χ^2 (3F:1NF)	P value
		Fle-cked	Non-flecked			
NP852/C591	P_1	0	10	10		
(M8)	P_2	12	0	12		
NP852/ C591	F_1	10	0	10		
(M8)	F_2	157	62	219	1.28	0.20-030
NP846	P_3	0	8	8		
NP846/C591	F_1	9	0	9		
(M8)	F_2	67	29	96	1.388	0.20-0.30

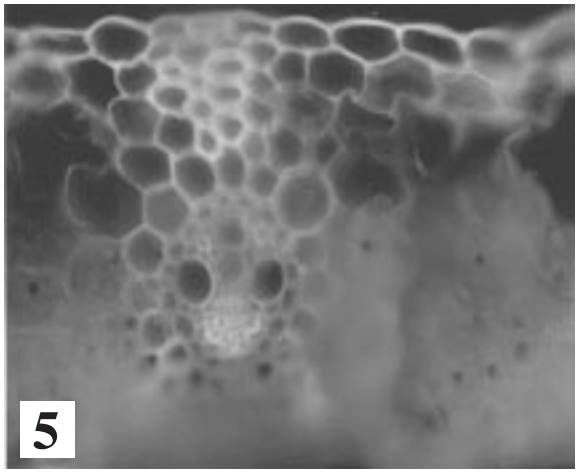
influence of flecking on a few yield related traits. Observations on the cross sections of leaf sheath from the normal and mutant plants under light microscope (without any staining) revealed clear differences in the level of greenness of mesophyll cells (Figs. 3 & 4). The mesophyll cells between the vascular bundles are filled with chlorophyll in the sections of the normal plants and in some sectors of mutant plants which may be the green area of the generally flecked leaf sheath. But the flecked area could be clearly distinguished where the mesophyll cells are distinctly empty with very few green patches. Epidermis and vascular bundles and other parenchymatous cells were not found to be affected in the flecked patches. Callose deposition was not observed in both normal and mutant plants under fluorescence microscope. The fluorescence seen in figures 5 and 6 is due to autofluorescencing vascular bundle tissues. Scanning florescence microscopy of normal and mutant plants in cross sections revealed a clear difference in stomata and surface deposit between the normal and mutant plants (Figs. 2 & 3). Also surface deposits were distributed irregularly in the leaf section of the mutant. Many chlorophyll deficient mutants studied earlier were found to show defect in the ultrastructure of chloroplast [12]. Since no ultrastructural studies were carried out in the present investigation, such possibilities can not be ruled out.

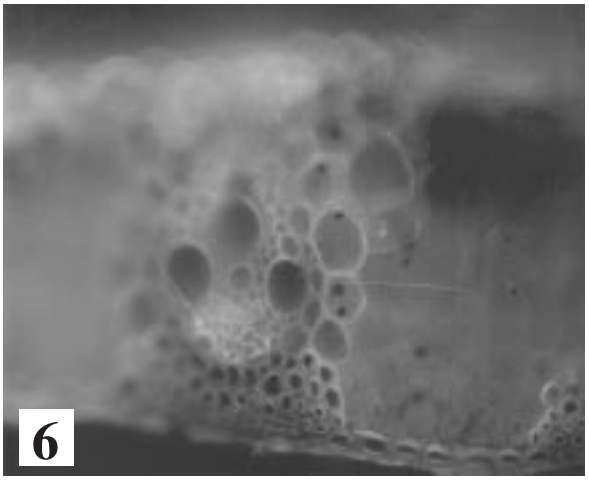
Chlorophyll deficient mutants have been reported in other crops earlier were observed to be recessive [5, 13] unlike the flecking mutant which is dominant. Exceptionally, mutants called disease lesion mimics (*Les*1 etc.) have been reported in many plants, a large number of them in maize and majority of them behave as dominant mutations that are developmentally programmed [7-8]. Johl *et al.* [15] identified quantitative trait loci (QTL) controlling differential expression of lesion











Figs. 1-6. (1) Field view of mutant C591 (M8) plants; (2) parents and F_1; (3 &4) Cross section of unstained mutant and normal leaf sheath under light microscope, respectively (Arrows indicate empty and chlorophyll filled mesophyll cells): (5 & 6) Cross section of aniline blue stained normal and mutant leaf sheath under incident light fluorescence microscopy, respectively.

Table 2. Segregation in F_2 generation derived from F_1 monosomic and disomic hybrids between monosomic series of var. Chinese Spring (CS) and C591 (M8) for flecking

Cross	Flecking	Normal	Total	χ^2 (3F:1N)*	P value
CS1A/C591(M8)	27	42	69	47.30	<0.001
CS1B/C591(M8)	81	23	104	0.46	050-030
CS1D/C591(M8)	19	7	26	0.06	0.80-0.70
CS2A/C591(M8)	53	35	88	2.36	0.20-0.10
CS2B/C591(M8)	47	11	58	1.14	0.30-0.20
CS2D/C591(M8)	22	12	34	1.95	0.20-0.10
CS3A/C591(M8)	56	25	81	1.48	0.30-0.20
CS3B/C591(M8)	65	44	109	13.72	<0.001
CS3D/C591(M8)	36	8	44	1.09	0.30-0.20
CS4A/C591(M8)	43	10	53	1.06	<0.001
CS4B/C591(M8)	19	59	78	87.70	<0.001
CS4D/C591(M8)	44	71	115	82.77	<0.001
CS5A/C591(M8)	22	59	81	96.96	<0.001
CS5B/C591(M8)	29	56	85	75.76	<0.001
CS5D/C591(M8)	18	34	52	45.22	<0.001
CS6A/C591(M8)	-	-	-	-	-
CS6B/C591(M8)	29	66	95	100.20	<0.001
CS6D/C591(M8)	38	9	47	0.85	0.50-0.30
CS7A/C591(M8)	32	10	42	0.31	0.70-0.50
CS7B/C591(M8)	51	61	112	51.85	<0.001
CS7D/C591(M8)	12	30	42	48.30	<0.001
C591(M8)/CS	104	28	132	1.01	0.50-0.30
Pooled excluding CS 1A/ C591(M8)	743	672	1415	98.46	<0.001

*F = Fleck, N = Normal; (χ^2 = 3.841, P=0.05)

mimics in specific genetic background. Intriguingly, more than half of the disease lesion mimic mutants inherit in a partially or completely dominant fashion, making them the largest class of gain-of-function mutations in maize. [15]. Koch *et al.* [16] reported that wheat cultivar Apogee produce flecking on leaf sheath and blade, which is helpful in identifying highly bunt susceptible plants.

All the monosomic F_1 hybrids, (CS monosomic series X C591 (M8)) were identified cytologically and scored for the presence of flecks. Variations in number and intensity of flecks were observed in monosomic F_1s as some of the monosomic F_1 expressed very poor flecking, while some showed fully developed flecks. The expression of lesions result from aberrations in all sorts of biological processes, with loss of cellular homeostasis (stable equilibrium within the cell) a common feature that eventually results in the death of affected cells [9].

The F_2 individuals in some of the crosses did not follow any Mendalian pattern of inheritance and produced aberrant segregating ratio. However, many of the F_2 populations segregated in 3 flecked : 1 non-flecked and followed disomic inheritance as observed in disomic x disomic cross. Flecking, exhibiting a characteristic brown-to-orange flecks has been observed in the aneuploids of group 5 chromosomes or the long arm of group 5 chromosomes in wheat [2]. This type of flecking may be due to reduction in dosage of genes for normal development located on the long arms of all group 5 chromosomes and is expressed under cool conditions in the absence of one of these six chromosomes. However, the flecking in C591(M8) is not temperature dependent but stable, developmentally programmed, dominant mutation under monogenic control resembling pathogenic attack and starts appearing only from boot leaf stage of the plant [11]. The aberrant behaviour

recorded in many monosomic F_1 derived F_2 populations is likely due to aneuploidy, genetic background or the presence of suppressors in those chromosomes of Chinese Spring in which distorted Mendelian genetic ratios have been observed. In such cases the homozygosity of the allele is necessary for full expression of flecks. The expression of disease lesion mimic mutant (*Les*1) is affected by genetic background, temperature, developmental age and wounding on necrotic spots in maize [17]. One or more than one of the above mentioned genetic phenomena might have created hindrance in identifying a critical line. Therefore, location of gene controlling flecking trait could not be precisely done and it is suggested that another monosomic series should be used for location of flecking gene. Alternatively, linked molecular markers can be identified in disomic x disomic crosses.

References

1. **Neatby K. W**. 1933. A chlorophyll mutation in wheat. J. Heredity, **24**: 159-162.
2. **Law C. N., Snape J. W. and Worland A. J.** 1987. Aneuploidy in wheat and its uses in genetic analysis. *In*: Wheat Breeding, (ed. F.G.H. Lupton). University Press Cambridge, Chapman and Hall Ltd., U.K.
3. **Pettigrew R., Driscoll C. J. and Rienits K. G.** 1969. Spontaneous chlorophyll mutant in hexaploid wheat. Heredity, **24**: 481-487.
4. **Williams N. D., Joppa L. R., Duysen M. E. and Freeman T. P.** 1985. Inheritance of three chlorophyll deficient mutants of common wheat. Crop Sci., **25**: 1023-1025.
5. **Klindworth D. L., Williams N. D. and Duyesen M. E.** 1995. Genetic analysis of chorina mutants of durum wheat. Crop. Sci., **35**: 431-436.
6. **Nair Sudha K.** 1998. Inheritance of flecking, phenol color reaction and solid stem in wheat. M.Sc. Thesis, Division of Genetics, Indian Agricultural Research Institute, New Delhi.
7. **Johal G. S., Hulbert S. H. and Briggs S. P**. 1995. Disease lesions mimic of maize: a model for cell death in plants. Bioessays, 17: 685-692.
8. **Walbot V., Hoisington D. A. and Neuffer M. G.** 1983. Disease lesion mimic mutations. Genetic engineering of plants. Plenum Pub. Crop, 431-442.
9. **Lorrain S., Vailleau F., Balague C. and Roby D.** 2003. Lesion mimic mutants: keys for deciphering cell death and defense pathways in plants. Trends Plant Sci., **8**: 263-271.
10. **Kumar Rajendra.** 1984. Induced mutagenesis for rust resistance in wheat. Ph.D. Thesis, Division of Genetics, IARI, New Delhi.
11. **Nair Sudha K. and Tomar S. M. S**. 2001. Genetical and anatomical analysis of a leaf flacking mutant in *Triticum aestivum* L. Euphytica, **121**: 51-58.
12. **Washington W. J. and Sears E. R.** 1970. Ethyl Methan Sulfonate-induced chlorophyll mutation in *T. aestivum*. Can. J. Genet. Cytol., **12**: 851-859.
13. **Freeman T. P., Duysen M. E. and Williams N. D.** 1987. Effect of gene dosage in light harvesting chlorophyll accumulation, chloroplast development and photosynthesis in wheat. Can. J. Botany, **65**: 2118-2123.
14. J**ohal G., Penning B. and Mcmullen M. D**. 2003. Identifying a natural suppressor of cell death in maize: Implications for gene discovery, diversity evaluation and beyond. Maize Genetics Conference. Paper No. 207. P. 141.
15. **Johal G. S.** 2007. Disease lesion mimic mutants of maize. http://www.apsnet.org/online/feature/mimics/
16. **Koch E., Weil B. and Eibel P**. 2004. Development of leaf symptom-based screening method for seed treatments with activity against Tilletia carries and application method using microbial antagomists. Zeitschrist-fur-Pflanzenkrankheiten-und-Pflazenschutz., **111**: 470-483.
17. **Hoisington D. A. Neuffer M. G. and Walbot V**. 1982. Disease lesion mimics in maize. I. Effect of genetic background, temperature, developmental age and wounding on necrotic spot formation with *Les* 1. Dev. Biol., **93**: 381-388.