The use of in vitro methods for blackpoint control and resistance in durum wheat (T. turgidum L. var. durum)

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The use of *in vitro* methods for blackpoint control and resistance in durum wheat (*T. turgidum* L. var. *durum*)

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SUMMARY - Blackpoint could be made to appear on durum seed in response to humid mist conditions. In such conditions the cell wall polyphenol levels of immature or ripe kernel tissues are modified. Moreover, under *in vitro* abiotic confined conditions, blackpoint-like symptoms can develop even on autoclave sterilized mature seeds. This response is related to the blackpoint field susceptibility of genotypes. No hypersensitivity phenomenon could be related to blackpoint.

Key words: Durum wheat, blackpoint, kernel quality, polyphenol, breeding.

RESUME - *"Utilisation de méthodes in vitro pour le contrôle de la moucheture et la résistance chez le blé dur". Il a été possible de faire apparaître à volonté la moucheture du grain de blé dur grâce à la réalisation de conditions de brumisation au champ. Dans ces conditions, le niveau de polyphénols pariétaux du grain est modifié. De plus, en conditions abiotiques confinées in vitro, des symptômes proches de la moucheture peuvent se développer même sur des grains mûrs stérilisés à l'autoclave. L'intensité de la réponse apparaît liée à la sensibilité au champ des génotypes à la moucheture. Aucun phénomène d'hypersensibilité du blé dur n'a pu être mis en relation avec la moucheture.*

Mots-clés : *Blé dur, moucheture, grain, qualité, polyphénol, sélection.*

Introduction

Blackpoint is typically observed as brown or blackish spots affecting all types of wheat kernel tissue. Embryo and pericarp coloration does not affect the quality of semolina. On the other hand, endosperm affected by blackpoint results in specks in semolina products. It seems that blackpoint occurs frequently in bread wheat. However, the milling process grinds bread wheat in smaller aggregates than semolina, thus reducing financial loss caused by blackpoint in bread wheat products. Durum wheat blackpoint is presently considered one of the most frequent crop accidents. French production in 1989 estimated around 3 million US dollars damage due to blackpoint.

The biochemical nature of this accident is not clear (Rossi *et al.*, 1992). Some biochemical results rule out the hypothesis of the occurrence of Maillard browning reaction (Vidal, 1974). Other references underline the importance of polyphenolic compounds in the blackpoint such as ferulic acid or its derivatives (Tabusse, 1986). Furthermore, several biotic and abiotic blackpoint factors were investigated, but it is not clear whether they are caused by direct, indirect or correlated factors (Maloy and Specht, 1988).The development of *Alternaria* and *Thrips* pathogens during anthesis period is often associated with the development of blackpoint. Humid atmospheric conditions since flowering period are equally associated with blackpoint development (Conner and Davidson, 1988; Conner *et al.*, 1990). Blackpoint resistance is actively bred, but at present we depend on natural field conditions to evaluate breeding lines. However, information from field experiments indicates that there is a relatively consistent classification of check genotypes for blackpoint occurrence. In these conditions, immunity is never observed. Durum wheat growers, breeders and the semolina and pasta industry are not able to predict or control this accident.

During the 1990-1993 period, we tried to find further answers to some of the basic questions concerning durum blackpoint:

(i) Can we generate field conditions which produce blackpoint?

(ii) Could we determine any biochemical, cytological and histological data associated with blackpoint in spotted mature kernels as well as in developing seeds of resistant and susceptible genotypes in conditions which did or did not lead to blackpoint?

(iii) Coloured, oxidized phenolic compounds are often implied in hypersensitivity necrotic reactions. Mycelia of various pathogenic fungi are frequently observed in blackpointed durum kernels. Could this type of host pathogen interaction be related to durum plants reactions when submitted to various hypersensitivity elicitors?

(iv) Is it possible to build an *in vitro* model developing blackpoint symptoms on excised developing or mature kernels under aseptic conditions?

Material and methods

Genotypes

All the studies we conducted in 1990, 1991, 1992 were made with the French durum varieties: Arbois and Néodur (susceptible to blackpoint) and Arcour and Primadur (resistant to blackpoint).

The effect of artificially humid atmospheric conditions on the development of blackpoint in durum crop

This experiment was aimed at determining the causes of blackpoint, and also at collecting blackpointed samples for biochemical and histochemical studies.

Samples were obtained at different stages of kernel development in order to observe the evolution of phenolic fractions. Mist conditions were obtained during the growth of kernels at Nîmes (Gard) under Mediterranean conditions. However, the 1992 spring season was very humid and it was impossible to differentiate between genotypic effects and damage generally not associated with blackpoint (*Fusarium*, brown kernel colour).

Biochemical and histochemical studies

Non soluble parietal fractions and soluble phenolic fractions were extracted from blackpointed areas of the pericarp and endosperm (generally around the grain furrow) of mature and growing kernels and analyzed using HPLC. Fluorescence micrographs with specific polyphenol colorations were obtained in the furrow area on whole kernels under the same experimental conditions.

Hypersensitivity studies

A variety of elicitor substances were tried on the cultivar Arbois seedlings: 13 fungi extracts without lipids, filtrates of 6 diverse parasitic fungal cultures, filtrates of 6 fungi species isolated from wheat.

In vitro modelization of blackpoint

Various *in vitro* embryo culture techniques were experimented on excised and whole growing or mature seed.

Results

Blackpoint development under mist conditions

Good reproduction of blackpoint development and levels of tolerance was obtained during 2 years (Table 1).

Table 1. Relation between mist and field blackpoint development. Blackpoint is computed as % of blackpointed seeds

Variety	Check 1991	Mist 1991	Check 1992	Mist 1992
Arcour	0.5	5.0	1.1	3.0
Primadur	0.2	4.2	4.7	3.9
Arbois	0.6	5.3	15.3	12.8
Neodur	1.2	9.6	6.6	9.6

Biochemical and histochemical results

Mature blackpointed kernels: The main insoluble polyphenols associated to the cell wall are ferulic acid, p-coumaric acid, diferulic acid, C-glycosylflavones, and the main soluble polyphenols are hydroxycinnamic derivatives, benzoic derivatives, free ferulic acid, C-glycosylflavones. Cell wall-associated ferulic acid was found to be much higher in blackpointed durum wheat (5.8 mg g^{-1} for blackpointed kernel bran against 0.95 mg g^{-1} for normal kernel bran).

Growing seed: The peaks of cell wall-associated ferulic and p-coumaric acid levels appear earlier for kernels grown under mist conditions. Histofluorescence data confirm polyphenol compound differences between blackpoint resistant and susceptible genotypes at the early milk stage.

Hypersensitivity to different elicitors

No consistent response at plantlet stage was found for any elicitor studied.

In vitro modelization of blackpoint

The preliminary results indicate that under various *in vitro* conditions, whole kernels at different stages (early milk stage to maturity) can develop blackpoint-like symptoms related to genotypic field blackpoint reaction. The main difficulty experienced was related to controlling aseptic conditions control. The autoclave sterilization of mature seed (121°C, 10 min) was very effective and did not stop the development of blackpoint-like spot of the devitalized seeds implanted on hydrated gelose *in vitro* medium. Genotype effects were highly significant and corresponded to official French field test trials (GEVES, 1992) (Table 2).

Table 2. Black spot intensity of appearance on mature durum wheat kernels on gelose medium

Cultivar	Intensity of spotting	Class of significance (Duncan test)	GEVES evaluation of blackpoint resistance
Arbois	3.87	a	4
Néodur	2.79	b	4
Primadur	2.07	c	7
Arcour	1.92	d	8

Discussion and conclusion

The first stage of this research was to determine whether we were able to obtain sufficient levels of blackpoint under field conditions in order to obtain more information concerning blackpoint in developing durum wheat kernels. The response to mist is clearly positive. However, it is difficult to establish blackpoint in dry windy weather ("mistral") or in wet weather if other diseases (i.e. *Fusarium sp.*) develop. This does not mean that moisture is a direct blackpoint factor; at this stage, one could suppose that moisture might lead to the development of some pathogenic organism inducing blackpoint. However, no elicitor of hypersensitive reaction was found which could consistently induce necrosis on durum wheat plantlets. Moreover, under completely aseptic *in vitro* conditions (for example after having submitted the growing medium and the seeds to autoclave sterilization), blackpoint-like symptoms developed in humidity saturated petri dishes. This indicates clearly that humid confined conditions could be implicated in blackpoint development in the absence of ordinary living pathogenic organisms, and even in the absence of living kernel cells.

Moreover, blackpoint type intensity reaction of mature kernels submitted to *in vitro* conditions after autoclave sterilization is highly related to blackpoint field reaction for the different genotypes (Table 2). Soluble or cell wall-related polyphenols varied during the seed development period in our mist and genotype experiment. It is not yet clear if a general reaction mechanism at the milk stage (for example 55% seed water content) could be easily analyzed in terms of histochemistry, or of biochemistry (cell wall associated ferulic acid content). At the mature stage the biochemical and genetical differences in blackpoint reaction could be more difficult to observe due to complex polymerization cell wall association phenomena and events affecting cell membranes, for example polyphenol decompartimentation.

Our joint series of experiments helped to establish the fact that blackpoint-like symptoms could be provoked in humid mist field conditions and even more easily *in vitro* in sterilized seed under moisture saturated confined petri dishes. The complex events leading to differential varied polyphenol accumulation patterns on maturing cell parts and tissues of the furrow region remains unclear. Nevertheless, we had the opportunity of developing several cheaper and safer tests for controlling blackpoint resistance.

These tests might help us to determine more specifically whether blackpoint is a unique physiologic reaction to certain abnormal growing and maturing conditions or whether various types of blackpoint development stem from the specific environment and genetic constitution of wheat.

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