

The Role of Total Nitrogen Content of Leaves in Temperature Sensitivity of Barley Cultivars to *Puccinia hordei* and Changes in Nitrogen Content Occurring During Development of the Disease

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ABSTRACT

The total nitrogen content of three barley cultivars: Cehada Capa (resistant at 25°C), CI 1243 (resistant at 5°C) and Rika 1 (susceptible at all temperatures) to *Puccinia hordei* was determined and compared in healthy and infected plants at 5° and 26°C. The results indicate that the total nitrogen content of cv. Cehada Capa leaves was greater than that of CI 1243 and Rika 1 and that of CI 1243 was greater than that of Rika 1 when plants were grown at 26°C. When plants were grown at 5°C, the percentage of total nitrogen content of leaves of cv. Cebada Capa was significantly greater than that of CI 1243 and Rika 1, but no differences were found between Rika 1 and CI 1243. It was concluded that the total nitrogen content has no role in the temperature sensitivity of barley cultivars. In most cases, especially in susceptible cultivars, the nitrogen content of infected leaves was greater than that of healthy plants when plants were incubated at 26°C, but there were no significant differences between total nitrogen content of infected leaves and that of their healthy control when plants were incubated at 5°C.

Keywords: Barley cultivars , Total nitrogen content , *Puccinia hordei* .

INTRODUCTION

It has been suggested that the temperature sensitivity of barley cultivars, especially cv, CI 1243 (carrying the Pa 9 gene) which is resistant at 5°C but susceptible at 25°C, might have a nutritional rather than a toxicological explanation [3], Although a relationship was found between the water soluble carbohydrate content of barley leaves and their susceptibility to *P. hordei*, it was concluded that sugar may

not be the only factor which has a role in the resistance or susceptibility of barley cultivars [3]. Widdowson *et al.* [12] found that the severity of barley brown rust was increased with fertilizer nitrogen. Wolfgang [13] could not find any significant correlation between nitrogen content and susceptibility or resistance of barley varieties to yellow rust. A review of the reports of many investigators [1,2,5], generally indicates that a high nitrogen application increases the susceptibility of

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paints to plant pathogenic fungi.

In order to gain some insight into possible nutritional factors, the total nitrogen content of barley leaves in healthy plants and plants infected by *P. hordei* was examined. The aims of this investigation were therefore to study:

- a) The effect of high (more than 25°C) and low (5°C) temperatures on the total nitrogen content of barley leaves, and
- b) The effect of infection by the three races of *P. hordei* on the total nitrogen content of barley cultivars.

MATERIALS AND METHODS

The original stocks of seeds of barley cultivars Cl 1243, Cebada Capa and Rika I and also original stocks of uredospores of three races, namely BRS 76.12, BR/EST and F of *P. hordei*, which were used throughout this investigation, were provided by Dr. Brian Clifford of the Welsh Plant Breeding Station, Aberystwyth.

Two experiments were conducted, in both experiments, the seeds of three barley cultivars were grown in plastic pots, containing John Innes compost No. 2, in a growth room at a constant temperature of 26°C. The intensity of light was 150 microeinsteins $m^{-2} sec^{-1}$ at the soil surface during the 16 h photoperiods.

The leaves of barley cultivars were rubbed lightly between moistened fingers to remove surface wax cuticle and thus increase the chance of penetration. The plants were inoculated with a mixture of five parts of talc and one part freshly harvested uredospore, using a small paint brush, then sprayed with water and covered with a polythene bag. After 4<S h, the bags were removed.

In experiment one, the healthy control and inoculated plants were incubated at 26°C and the leaves of the plants were harvested nine days after inoculation. In the second experiment, plants were incubated at 5°C and leaves were harvested 3S days after inoculation and determined for their total

nitrogen content.

The healthy and infected plants to be used for determination of nitrogen were washed carefully with distilled water in order to wash away spores, tale (which was used for inoculation) and any foreign materials, e.g. soil. Before harvesting, the water which was used for washing was allowed to evaporate from the surface of the plants. Care was taken to harvest all samples in each experiment at the same time of the day.

After cutting, the samples were placed in polythene bags, freeze-dried to constant weight, ground in a micro hammer mill and then passed through an 80 mesh sieve. Ground samples were thoroughly mixed, placed in glass bottles and freeze-dried again to constant weight. The bottles were tightly capped and kept for analysis [8].

The micro-Kjeldahl method [10] was used for determination of total nitrogen. 0.1 g dried sample was weighed into a 30 ml kjeldahl flask, followed by 2.5 g kjel tablets (Thomson and Capper Ltd. Liverpool). Six millilitres of concentrated sulphuric acid were then added. The flask was then placed over a small flame on a kjeldahl digestion rack and heated until frothing ceased. Digestion was for one hour after frothing had ceased. One blank determination using 100 mg of sucrose in place of the sample was carried out. Distillation was performed using a Markham distillation apparatus and Ulration was made against 0.1)1 MH₂SO₄.

A completely randomized design with four replications was used in each experiment. The means were compared using Duncan's multiple-range test with LSD as described by Little and Hills [4].

RESULTS

The mean percentages of total nitrogen content of healthy and rust-infected leaves of barley when plants were incubated at 26°C are presented in Table 1. The results indicate

Table 1. The mean^a percentages of total nitrogen content (dry material) of healthy and rust-infected leaves of barley (*P. hordei*) nine days after inoculation when plants were incubated at 26°C

Cultivars	Infected			Healthy
	Race F	Race BRS 76.12	Race BR/FST	
Cebada Capa	A 3.4 a AB	A 3.24 a	A 3.25 ab	A 3.6 a A
CI 1243 Rika 1	2.95 ab B	AB 3.20 a	B 3.41 b	2.7 a
	2.64 b	B 2.72 a	B 2.81 a	A 2.192 c

Significant differences are denoted by different small letters within each column and by capital letters within each row.

^a Mean values are the average of four replications and two samples of each replicate and three determinations for each sample.

that the percentage of total nitrogen content of healthy leaves of cv. Cebada Capa was significantly greater than that of CI 1243 and Rika 1 and that of CI 1243 was greater than that of Rika 1.

There were no significant differences between the percentage of total nitrogen content of leaves on cv. Cebada Capa infected with three different races and that of the healthy control. In most cases, the percentage of total nitrogen content of leaves of cv. CI 1243 and Rika 1 infected with three different races of *P. hordei* was greater than that of the healthy control. The details of these results are shown in Table 1.

Total nitrogen content of healthy plants and those infected with *P. hordei* when plants were incubated at 5°C are presented in Table 2. There were no significant differences between the total nitrogen content of leaves of three barley cultivars infected with three races of *P. hordei* and their healthy controls when plants were incubated at 5°C.

DISCUSSION

Results of experiments carried out at 5°C and 26°C (Tables 1 and 2) clearly indicate that the percentage of total nitrogen content of leaves of Cebada Capa was greater than

that of CI 1243 at both temperatures. Total nitrogen content of healthy leaves of cv. Rika 1, which was temperature insensitive and susceptible at all temperatures, was less than that of healthy leaves of Cebada Capa and CI 1243 at 26°C.

As has been mentioned previously, Cebada Capa and CI 1243 are resistant and susceptible at 26°C, respectively and *vice versa* at 5°C. If the total nitrogen content has any role in resistance and susceptibility or temperature sensitivity in these two barley cultivars, the reversal of resistance and susceptibility from one temperature to another should therefore be accompanied by a corresponding reversal in nitrogen content. This phenomenon did not happen in this case. On the other hand, since cv. Rika 1 is temperature insensitive and susceptible at both temperatures, the total nitrogen content of healthy leaves of this cultivar should not change when plants are grown at 5°C or 26°C. However, as the results show, the percentage of total nitrogen content of healthy leaves in this cultivar at 26°C was 2.19 and at 5°C was 4.86. It can be concluded therefore that total nitrogen content plays no role in resistance / susceptibility or temperature sensitivity.

These results are in agreement with results of experiments carried out by Wolfgang [13]

Table 2. The mean percentages of total nitrogen content (dry material) of healthy and rust-infected leaves of barley cultivars 38 days after inoculation when plants were incubated at 5°C

Cultivars	Infected			Healthy Control
	Race F	Race BRS 76.12	Race BR/EST	
CebaUa Capa	A 5.83 a	A 5.8 a A	A 5.59 a	A 5.65 a
CI 1243 Rika I	A 5.24 ab	4.81 b	A 5.20 a	A 4.82 b
	A 5.03 b		A 5.10 a	A 4.86 b

Significant differences are denoted by different small letters within each column and by capital letters within each row. (-) Rika I leaves infected with race BRS 76.12 were not analysed because the material were not sufficient.

who reported that there was no significant correlation between nitrogen content and susceptibility or resistance of barley varieties to yellow rust. Widdowson *et al.* [12] found that the severity of barley brown rust was increased with fertilizer nitrogen.

The nitrogen content of barley leaves infected with *P. hordei* when incubated at 26°C (Table 1) indicates that although in a few cases there were no statistically significant differences between percentages of nitrogen content of rust-infected leaves and those of the controls, the nitrogen content of infected leaves was always higher than that of the control in susceptible varieties (CI 1243 and Rika I). There were no significant differences between the total nitrogen content of leaves of Cebada Capa infected with the three races and the healthy control. These results are in agreement with the report of Shaw and Colotelo [7] and Shaw [6] who found that with rust infection on the susceptible wheat variety Little Club, total nitrogen increased as the rust developed, but a decrease in total nitrogen occurred with infection on the resistant wheat variety Khapli.

Some investigators have attempted to interpret the change in protein composition and nitrogen content of rust-infected leaves. For example, Uritani and Stahmann [11] stated that in response to an infection, the

respiratory rate is increased in many host plants. They assumed that the augmented respiration is coupled to adenosine triphosphate utilizing systems such as protein synthesis.

The total nitrogen content of rust-infected leaves when plants were incubated at 5°C (Table 2), indicates that although the percentage of total nitrogen of infected leaves was, in most cases, greater than that of the control, still these differences were not statistically significant.

It may be suggested that the increase in the nitrogen content of leaves after inoculation of rust at 5°C is less than that of leaves incubated at a high temperature. Staples and Stahmann [9] reported that the protein content of rusted susceptible bean leaves was not grossly different from that of healthy leaves even after extensive rust growth (they incubated the plants at a normal temperature).

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نقش میزان کل ازت برگها در حساسیت به درجه حرارت ارقام مختلف

جو نسبت به قارچ *Puccinia hordei* و تغییرات

مقدار ازت برگها در خلال توسعه بیماری

چکیده

میزان کل ازت برگهای سه رقم جو: Cebada capa (مقاوم در ۲۵ درجه سانتی‌گراد)، CI 1243 (مقاوم در ۵ درجه سانتی‌گراد) و Rika 1 (حساس به بیماری در همه درجات حرارت) نسبت به قارچ *Puccinia hordei* اندازه‌گیری گردید و مقدار ازت برگها در گیاهان سالم و گیاهان آلوده در ۵ و ۲۶ درجه سانتی‌گراد مورد مقایسه قرار گرفت. نتایج نشان داد، هنگامی که گیاهان در ۲۶ درجه سانتی‌گراد رشد کردند میزان کل ازت برگها در رقم Cebada capa بیشتر از میزان کل ازت برگها در ارقام Rika 1، CI 1243 می‌باشد و مقدار کل ازت برگهای CI 1243 بیشتر از مقدار ازت Rika 1 می‌باشد. ولی هنگامی که گیاهان در ۵ درجه سانتی‌گراد رشد کردند درصد کل ازت برگهای رقم Cebada capa بیشتر از Rika 1 و CI 1243 بود، اما از نظر آماری اختلافی بین میزان ازت CI 1243 و Rika 1 مشاهده نشده و نتیجه گرفته شده که میزان کل ازت برگها هیچ نقشی در پدیده حساسیت به درجه حرارت ارقام ندارد. موقعی که گیاهان با قارچ عامل بیماری مایه‌زنی شده و در ۲۶ درجه سانتی‌گراد نگهداری شدند در بیشتر حالات و به ویژه در ارقام حساس میزان کل ازت برگهای آلوده بیشتر از برگهای سالم بود. اما هنگامی که گیاهان مایه‌زنی شده در ۵ درجه سانتی‌گراد نگهداری شدند هیچ اختلاف معنی‌داری بین مقدار ازت برگهای مایه‌زنی شده و سالم وجود نداشت.