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ROLE OF NUTRITION IN EXCISE LEAF CULTURING OF WHEAT RUSTS

By

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Three sugars at different concentrations were compared in detached leaf culturing of wheat stem rust Effect of low and high level of sucrose in the medium as affecting the sporulation of five races of stem rust were also studied. Plant kinins such as benzimidazole and kinetin at different proportions were estimated separataly and in combination with sucrose. 10% sucrose with 100ppm denzimidazole was found to be the best medium for detached leaf culture of wheat rusts under Indian conditions.

INTRODUCTION

From the time of initiation of this method, it has been well emphasized that nutrition is the basic principle for successful culturing of rust on detached leaf culture. The importance of carbon nutrition in detached leaf culture was suggested as early as 1883 by Boehm, while Mains (1917) demonstrated that the development of rust is dependent upon the food supply of the host and not upon the host protoplasm. Waters (1928), Trelease and Trelease (1929), Yarwood (1934), Sempio (1950), Wu (1952), Browning (1954), Silvermann (1960), Prasanna Kumari and Wilcoxson (1970), Berly and Kozhevnikov (1974) studied and confirmed the utility of different sugars in detached leaf culture of rusts.

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The tendency of chlorophyll in cut leaves to disintegrate quickly was found to hinder the successful maintenance of rusts on detached leaves. Many workers have thrown light on the utility of adding plant kinins to the medium to prevent chlorophyll destruction and thereby to prolong the life of detached leaves. Trelease and Trelease (1929). Person et al. (1957). Dalton Wang et al. (1961), Samborski et al. (1961), Browder (1964),Wolfe and Macer (1966), Hermannsen (1966).Doling (1966),Hooker and Yarwood (1966). Prasanna Kumari and Wilcoxson (1970),Singh and Renfro (1971),Balazo and Kiraly (1972) and Atif and Wilcoxson (1975) had studied the applicability of analogues of nucleic acid components and other substances and found beneficial effect of certain substances in prolonging the life of the culture.

Here, in the study, the selection of a standard medium constituting a carbohydrate and a plant kinin was carried out for the successful culturing of wheat rust under Delhi conditions.

MATERIALS AND METHODS

Seeds of wheat variety Agra local were sown in 10 cm pots. At the primary leaf stage the leaves from the tip were inoculated uniformly with the rust inoculum from stem rust race 21 by means of an inoculator operated by vaccum pump. The pots were incubated for 24 hours in a humidity chamber and then kept outside on the glasshouse benches. As soon as the flecks were visible the leaves were harvested and cultured in incubators keeping the opimum temperature (20$\pm$3°C) and optimum light conditions (550 fc) for the normal development of stem rust of wheat. The uredo inoculum produced by the detached leaf cultures were collected by means of a collector operated by means of vaccum pump.

Sugars : For selection of sugars, preliminary studies were carried out in test tubes with five sugars with water as control. It is found that glucose, fructose and sucrose enhanced sporulation of the rust. Hence, in further studies

only glucose, fructose and sucrose were compared. One serious handicap of the test tube method is the difficulty in collecting the uredospores without spilling it in the medium and on the sides of the test tube. Considerable wastage of uredospores occurred and accurate recordings of the number of spores per lesion was thus not possible. Accordingly, the petriplate method (Hooker and Yarwood, 1966) was adopted in a modified form.

RESULTS

(*a*) *To estimate the optimum concentration of sugar for disc* : In preliminary studies, (Prasanna Kumari and Wilcoxson, 1970), a medium consisting of 2 ml of 10% sucrose and 50 ppm benzimidazole was found useful. Here, with a view to arrive at the optimum requirement of these sugars, viz, sucrose, fructose and glucose, different concentrations (5, 10 & 15%) of each of these sugars individually tested using the above-said medium as control. The experiment was replicated thrice.

The observations on pustule size and number of spores per pustule recorded after five to seven days of transfer of leaves to the different media are tabulated in Table 1.

In 5% solution of all the three sugars on the fifth day, sporulation of the infected leaves was almost the same. The number of spores produced per pustule in sucrose was the highest as compared to glucose and fructose. In sucrose, the production of spores continued for more than two weeks, while the leaves in glucose and fructose turned yellow after five to six days. In 10% fructose, the sporulation is much better than at 5% level, but immediate death of the leaves occurred due to infestation by contaminating fungi.

In 10% glucose, pustule development was poor and the leaf became chlorotic with water permeated areas spreading to the tip. Few pustules that developed sporulated after a long incubation period. The leaves were infected

with contaminating fungi and died in five to seven days. In fructose sporulation was better as the leaves remained greener few more days. But in sucrose not, only the general appearence was better than other two treatments, pustules were bigger and continued sporulation longer than in glucose or fructose. In 15% solution of all the three sugars, the leaves showed a tendency to become flaccid and then senescence occurred. The average sporulation in terms of size of the lesion and number of spore output was quite superior in the case of sucrose than in fructose or glucose.

Table 1. *Effect of three different concentrations of glucose, fructose and sucrose on sporulation of black rust race 21 of wheat in datached leaf culture*

Concentration (%)	Incubation period (days)	Pustule/ leaf	Lesion size (mm)	Spores/ pustule (X 10^5)
Glucose				
5	12	6.0	1.73	3.44
10	11	4.0	2.62	6.14
15	12	7.5	2.62	4.56
Fructose				
5	11	8.0	1.86	3.61
10	11	7.3	2.41	7 11
15	10	7.3	1.80	5.56
Sucrose				
5	9	4.0	1.46	4.65
10	8	6.4	5 27	8.97
15	9	6.1	3.23	7.79
Control	8	6.5	4.58	8.22

Analysis of Variance
Effect of different sugars

Source of variation	d.f	M.S.S.	F
a. Lesion size			
Treatments	9	57.599	13.85**
Error	140	4.156	
b. Spore count			
Treatments	19	72.425	23.17**
Error	440	3.125	

** Significant at 5% and 1% levels

The best performance of the sporulating pustule was in control (10% sucrose + 50ppm benzimidazole). In this the infected leaves remained fresh and greener for several days than in sugar solutions. This experiment revealed that among different sugars tested, sucrose at 10% level was found significant over other medium of control as regards lengevity and vigour to the detached leaves. But10% sucrose alone was less effective as compared to combi nation sugars

(b) *Sporulation of certain races of black rust of wheat as affec ted by differences in levels of Sucrose in this medium* : In the experiment, effect of two concentrations of sucrose (viz. 2% and 10%) on five reces of stem rust of wheat (viz. 40, 21, 34, 15, 117-A) was studied. The experiment was replicated thrice. The results are tabulated in Table 2.

Table 2 *Effect of 2% and 10% concentrations of sucrose on sporulation of different races of black rust in detached leaf culture*

	2% Sucrose					10% Sucrose				
	Races									
	40	21	34	15	11-A	40	21	34	15	117-A
Incubation period (days)	12	11	12	14	16	9	7	8	9	13
Pustule/leaf	2.0	1.8	2.0	1.5	028	4.5	4.0	6.0	3.2	0.6
Lesion size (mm)	0.61	0.70	0.47	0.51	0.30	2.31	1.79	1.71	1.44	0.70
Spores/pustule ($x10^5$)	0.92	1.06	0.96	0.10	0.70	8.48	6.74	7.95	6.18	0.70

Analysis of Variance

Effect of 2% and 10% sucrose on different races

Source of variation	d.f.	M.S.S.	F
a. *Lesion size*			
Families	9	22.307	
Between treatments	1	132.845	256.59
Batween family/treatmentents	8	8.490	16.39
Error	440	0.517	
b_1. *Spore count at 2% sucrose*			
Treatment	4	1.927	11.25**
Errors	70	0.171	
b_2. *Spore count at 10% sucrose*			
Treatment	4	145.469	11.50*
Error	70	12.645	

** Significant at 5% and 1% levels

In 2% sucrose solution, the active life of the leaf was found to be short although the leaves looked fresh and green for more than seven days. The pustules were smaller in size, had a withered look and were less flourishing than in 10% sucrose. A tendency to from teleutospores was found in most of the pustules after two weeks which invariably terminate the life of the rust. Viability of the uredospores was found normal (80-100%). Leaf tissue in the vicinity of the pustules showing teleutospores were green and alive tor a longer time, probably because the rust was not in a vigorous developmental phase and thus not depleting the leaf. As regards sporulation, the different races behaved differently in the two concentrations of sucrose. However, in 10% sucrose solution the performonce of all the races was significantly better than in 2% sucrose solution. The incubation period of these races was shorter by 3-5days in 10% (7–13 days) than in 2% sucrose (11-16 days). Race 117-A was found to be the weakest among these races cultured in both the concentrations of sucrose.

(c) *To select a suitable concentration of Benzimidazole for incorporating in the standard medium* : Different concentrations of benzimidazole (10-1000 ppm) were tested to obtain the optimum concentration for incorporating in the medium. Distilled water was used as control. The number of pustules, size of pustules and number of spores per lesion recorded on the seventh day are tabulated in Table 3.

Table 3 *Effect of nine concentrations of benzimidazole on sporulation of stem rust race 21 in detached leaf culture*

Sl. No.	Concentration (ppm)	Incubatron period (days)	Pustule/ leaf	Lesion size (mm)	Spores/ pustule ($x10^5$)
1	10	14	3.2	0.56	0.09
2	20	14	3.2	0,63	0.12
3	50	13	5.2	1.00	0.26
4	75	12	4.5	1.06	0.24
5	100	2	4.8	1.43	0.41
6	200	14	5.4	1.03	0.21
7	500	15	3.2	0.59	0.14
8	750	15	3.0	0.32	0.04
9	1000	—	—	—	—
	Control (distrilled water)	14	2.7	0.40	0 05

Analysis of Variance
Effect of benzimidazol

Source of variation	d.f.	M.S.S.	F
a. *Lesion size*			
Treatment	8	5.877	47.25**
Error	387	0.124	
b. *Spore count*			
Treatment	8	0 214	12.98**
Error	126	1.651	

** Significant at 5% and 1% levels

Control in distilled water showed very poor sporulation. Developed pustules were few but the levaes showed yellowing from the third day. In 10 ppm solution, the leaves though looked better rhan in water, sporulation likewise was poor. At 20 ppm the pustules developed similar to 10 ppm, but average size of the pustule and average number of spores were slightly better than at 10 ppm. In 50 ppm, the total number of pustules developed was more than in 20 ppm and the sporulation also showed conspicuous differences. Leaves looked quite fresh with the characteristic greenness even on the seventh day although the production of spores was slow. In 75 ppm solution, the average number of spores as well as the average size of the pustules were almost similar to 50 ppm. In 100 ppm solution, the development of the pustules was better than other concentrations discussed so far. Sporulation was found to be increasing gradually as the concentration was raised from 10 ppm to 100 ppm, while there was a sharp fall in sporulation as well as in the vigour of the leaf as the concentration increased from 100 ppm to 750 ppm when it reached 1000 ppm, sporulation was almost nil. From this, it is obvious that 100 ppm gave the maximum sporulation and the life of the leaf in a normal state was also prolonged. The results show that 100 ppm concentration was significantly better than other concentrations and hence this was used in further studies.

(b) *Evaluation of concentrations of Kinetin to be incorporated into the standard medium :* Earlier reported work as well as preliminary trials showed that kinetin was needed only in smaller doses. So the concentrations 10, 20

40, 60. 80 and 100 ppm were tried against distilled water as control. Observations recorded after seven days are tabulated in Table 4.

Table. 4, *Comparison of six concentrations of Kinetin on sporulation of black rust race 21 of wheat in detached leaf culture*

Sl. No.	Concentration (ppm)	Incubation period (days)	Pustules/ leaf	Lesion size (mm)	Spores/ pustules ($x10^5$)
1	10	14	2.6	0.28	0.085
2	20	14	3.8	0.55	0 146
3	40	12	4.0	1.01	0.250
4	60	12	3 0	0.90	0 230
5	80	13	2.0	0.50	0.090
6	100	14	2.3	0.25	0.036
7	Control (distilled water)	14	2.3	0.25	0.049

The control in distilled water showed very poor sporulation. The sporulation in 10 ppm solution was only slightly better than in distilled water. The 20 ppm solution was found comparatively better than the control and 10 ppm solution as regards sporulation. The leaves looked definitely better than in 10 ppm and remained green for more than 10 days. Kinetin at 40 ppm, as is evident from the table was the best concentration tried regarding good development of pustule and good sporulation. Leaves remained green and fresh for more than two weeks. The concentration at 60 ppm was good but showed comparatively less spore production and pustule development as against 40 ppm At 80 and 100 ppm there was a sharp fall in the development of pustules and the number of spore produced by the pustule. In 100 ppm growth of the rust pustule was arrested and spore production was much adversely affected. The leaves showed flaccidity and dried up quickly. Thus 40 ppm kinetin solution was found to be good as far as maintenance of infested leaves in a vigorous state for longer periods and for maxmimising the output of spores.

As kinetin is more costly and not easily available Benzimidazole is used for standardisation of the cultural solution and further studies.

(e) *Combined effect of Sugars and Benzimidazole on sporulation;*

Sucrose and benzimidazole were combined in the following ways to study the effect of combination media on sporulation in disc,

1. 5% sucrose + 100 ppm benzimidazole
2. 5% sucrose + 1000 ppm benzimidazole
3. 10% sucrose + 100 ppm benzimidazole
4. 10% Sucrose + 1000 ppm Benzimidazele
5. 10% Sucrose

The observation after seven days are tabulated in Table 5.

Table 5. *Effect of combined medium on sporulation of black rust race 21 tested on wheat leaves in detached leaf culture*

Sl. No.	Medium Sucrose	Benzimidazole	Incubation period (days)	Pustule/ leaf	Lesion size (mm)	Spores/ pustule ($x10^5$)
1	5%	100ppm	10	3.8	1.27	6.25
2		1000 ,,	14	3.0	0.89	1.38
3	10%	100 ,,	8	4.5	2.58	9.63
4		1000 ,,	12	4.0	1.01	2.33
5	Control	10% sucrose only	8	4.5	2.08	7.70

Analysis of Variance

Effect of combined medium

Source of variation	d.f.	M.S.S.	F
a. *Lesion size*			
Treatment	3	27.345	49.66**
Error	176	0.550	
b. *Spore count*			
Treatment	3	285.908	18.35**
Error	16	2.688	

** Significsnt at 5% and 1% levels

From the table, it is obvious that in 10% sucrose + 100 ppm benzimidazole (treatment No. 3), the rust sporulated heavily when compared to other treatments. The cultures were in a very vigoreus condition even on the 17th days. This incidently was also the combination of the best (sucrose) and best (benzimidazole) concentrations when tried individually.

Five percent sucrose and 1000 ppm benzimidazole when combined together and the rust cultured, there was not much effect in sporulation. This treatment was the poorest as seen from the Table. The development of pustule itself was feeble. The leaves had a withered look, curling from the tip and the culture died within ten days. Treatment No. 1 is definitely better than the second treatment. The development of the pustules was normal, the cultures looked more vigorous and lived for several days. The fourth treatment with sucrose at 10% and benzimidazole at 1000 ppm is not as good as the first treatment. Although pustules developed, they were faint in colour showing less spore production. There was a general tendency of the leaves to wither and dry up after a week.

Third treatment with sucrose and benzimidazole at the optimum was significantly better than the control of 10% sucrose as regards sporulation as well as the life span of the leaf. The leaves in treatment No. 3 were fresh and living for about three weeks with pustules vigorously sporulating while in control, the leaves live hardly ten days in vigorous condition. Infestation by hyperparasites and moulds was high in 10% sucrose which brought about immediate death of the leaves whereas treatment No. 3 was almost free of contamination for a longer time. The number of developed pustules in the control in 10% sucrose was similar to tretment No. 3 showing that the development of rust was not at all impeded in this.

In view of advantages with treatment No. 3 (10% sucrose + 100 ppm d enzimidazole) and as it was found significant statistically it is adopted as the standard medium for further studies.

Discussion

In the current investigation althrough the rust developed in different concentations of sugars (5-15% glucose, sucrose ane fructose), the maximum pore output, as well as low rate of occurrence of saprophytes was recorded in 10% sucrose solntion. The leaves tended to be flaccid in higher concentrations of sucrose while they remained green and fresh for more than three weeks

in 10%solution. The excessive activity of the rust alone exhibited the senescence of leaves in this medium. A lower concentration of sucrose (2 percent as used successfully by Waters, 1928, in culturing different rust fungi) was comoared with 10 % sucrose with regard to sporulation of five races of stem rust. The conspicuously superior level of production of spores in 10% sucrose solution may be due to the fact tnat the peak period of sporulation was reached within few days and the spores daily released were heaped up on the pus tule. While in 2% sucrose the incubation period was longer and pustule dehiscence was delayed resulting in poor spore production by that time. Yarwood (1934) in his studies with clover leaf parasites also found 2% sucrose solution to be less effective than higher concentrations. Concentrations ranging from 10-16%° were found suitable by him for the development of these parasites.

The utility of plant kinins iike benzimidazole and kinetin and other metabolites was investigated at length by many workers. In the current studies an evaluation of 10 to 1000 ppm benzimidazole and 10 to 1000 ppm kinetin was made with reference to rust fungi of wheat. Although the host remained in a fresh and vigorous condition in benzimidazole, the rust showed only limited growth beyond 200 ppm. Hooker and Yarwood (1966), however, found that benzimidazole was effective in concentrations of 160-1240 ppm for culturing corn rust. This striking difference in the activity of benzimidazole was possibly due to change of the host and the rust, In all concentrations of benzimidazole unlike in sugar solution, there were certain prominent features, such as the leaves looked fresh and the incubation period was more as well as number of pustules developed was high. This may be due to the sluggish sporulation of the developed pustules which may not be depleting the leaf of its food immediately. Kinetin above 60 ppm inhibited the rust development though the cut-leaf thrived vigorously in all the concentrations tested. Although it is know that the vigour of the host effects the vigour of the rust there was some inhibitory mechanism resisting the development beyond certain concentrations of the plant kinins (Doling, 1966; Wolfe, 1966; Hermannsen, 1966; Samborski, 1961). Even the concentrations allowing maximum sporulation, considerably delayed pustule development and their dehiscence]

Accordingly the use of these plant kinins alone as the substrate for culturing wheat rusts did not serva the purpose. A 100 ppm benzimidazole solution and 40 ppm kinetin solution separately were found quite optimum in maintaining the host tissue as well as the parasite. Owing to ready availability, comparatively low cost and ease in preparation, benzimidazole was selected for incorporation with sucrose base for standardising the medium. After comparing different proportions of sucrose and benzimidazole for their effect on the vigour of the host and maximum spore output, 10% sucrose with 100 ppm benzimidazole solution was selected as the best combination for culturing the rust fungi on wheat under Indian conditions.

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