韓國作物學會誌 第15號(1974.)
J. Korean Soc. Crop Sci. Vol. 15 : 115-121

代謝沮害劑에 依한 "통일"벼 赤枯現象 誘發에 關하여

郭 炳 華·金 璨
高麗大學校 農科大學

Induction of Red Discoloration in Rice var. Tongil with Certain Metabolic Inhibitors

Kwack, Beyoung Hwa and Kim, Chan

College of Agriculture, Korea Univeraity

Abstract

Artificial induction of nitrogen deficiency symptoms(leaf chlorosis) with two root respiratory inhibitors(DNP and Na_2S) was studied and regarded to be the same characteristic to red discoloration in rice var. Tongil seedling leaves as well as adult ones. Tongil(IR 667) was shown to be more nitrogen sensitive and have more distinctive appearance of the leaf discoloration than Punggwang(a native *Japonica*-type varity.)

Conclusions were drawn from the present data that so-called red discoloration of Tongil under the natural field conditions is brought about either by insufficient nitrogen supply in soils or certain factors which may limit at any time the root absorption of nitrogen(low temperature, toxic gases or substances, poor drainage, around roots, etc.) in soils even with ample supply of it.

Introduction

Tongil(IR 667) is a new variety of rice plant developed in 1969 at the Office of Rural Development in cooperation with College of Agriculture, Seoul National University in Suwon in response to the government policy to increase the rice production in Korea. The variety is arousing a great interest in farmers as well as in the authorities concerned because it has been demonstrated to be an excellent variety with high yielding capacity, and it is to be widely grown throughout the country with the government cooperation[5,6,8,10].

However, red discoloration, which is a kind of physiological disorder, has been known to occur on the leaves of rice var. Tongil before long after having been introduced into some parts of the country, and this seems to appear at any time of the year while grown under an unfavorable cultural condition[5,6,9]. Thus another problem was presented in relation to a possible reduction of the yield, by inhibiting the formation and maintenance of chlorophyll in the leaves of rice var. Tongil.

Causes of the red discoloration and some preventive measures were elucidated first by Kwack and Koo[2].

A series of physiological experiments were carried out to find out the fundamental principles of red discoloration using relatively young rice plants this time, and indicated the fact that red discoloration of Tongil leaves is attributable to the deficiency of nitrogen in the leaves and to the factors limiting the nitrogen absorption of roots.

The present studies were to show that there is a conspicuous difference in chlorosis(red discoloration on mature plant of Tongil) and chlorophyll formation between Tongil and Punggwang(a native variety), in relation to nitrogen absorption from roots when certain metabolic inhibitors are administered.

Materials and Methods

Rice var. Tongil (IR 667, Suwon #214...Indica origin) and Punggwang(a recommended variety in Kyunggi Province...Japonica type) were used in this study to compare the extent of leaf discoloration. Metabolic inhibitors used were 2,4-dinitrophenol (DNP) and sodium sulfide(Na_2S) for the purpose of respiratory inhibitor in root systems [4], and ammonium sulfate,$(NH_4)_2SO_4$, was used as the source of nitrogen fertilizing.

Various levels of ammonium sulfate as 0, 50, 100, 150, 200, and 250mg/l were set up to see the effect of nitrogen supply on difference in appearance of the discoloration between the two rice varieties. Treatment of DNP was made at either 0, 2, 10, or 50mg/l, and that of sodium sulfide at 0, 10, 50, 100, 200, and 500mg/l. 250mg/l ammonium sulfate was equally contained in the various levels of DNP and sodium sulfide solution, and so mixed solution was used. All solutions of nitrogen compound and chemicals were made with tap water, and each treatment had three replications. 50 seeds of each rice variety were sown in a Petri dish of 12 cm in diameter containing 20 ml tap water, and they were germinated and grown at 30 °C in an incubator for a week until they reached 4 cm in height.

The extent of leaf discoloration in two varieties was observed from the leaves after the effect of chemicals was noticeably shown on the leaves(four or five days after treatment). The optical density [7] was measured with a spectrophotometer in order to see and compare the extent of leaf discoloration observed.

Results and Discussion

Rice var. Tongil(IR 667) developed at the Office of Rural Development, Ministry of Agriculture and Fishery, Korea a few-year ago, was introduced as a high yielding, disease resistant and anti-lodging variety. This variety is a true-bred hybrid made through triple crosses among Yukara(a Japanese variety...Japonica type), Taichung Native #1(a Taiwanese variety...Indica type), and IR-8(a Philippino variety...Indica type) [8,10,11].

It was reported that red discoloration of rice var. Tongil might be caused by an excess or deficiency of certain minor elements in soil, and by certain unfavorable conditions for growth as low temperatures, poor drainage, and post injuries, etc. [9,10]. However we still need accumulated experimental data to account for the cause of red discoloration in var. Tongil.

According to the report of Ahn [1], Kwack and Koo [2], and Kwack [3], the red discoloration appears on rice leaves at any time regardless of a specific period of growth in case that nitrogen deficiency occurs in the leaves of Tongil, i.e., insufficient nitrogen application or the restriction of root absorption caused by various factors such as low temperature, toxic gases or substances, poor drainage causing reduced respiration rate of roots, etc. The Japonica varieties such as Jinhung and Paldal led normal growth and appearance even under the conditions that red discoloration occurs in the rice var. Tongil owing to nitrogen deficiency, and they were observed to be less sensitive to nitrogen shortage than var. Tongil[1,2].

Different spectral peaks were found between var. Tongil and Punggwang when chlorotic pigments extracted from two varieties were compared spectrophotometrically; that is, Tongil had peak closer to red wave length than Jinhung[2]. Consequently the red discoloration of var. Tongil may be a particular phenomenon to appear like a kind of disease because orange or rather red pigments of Tongil leaves are uniquely showed to human eyes as a result of physiological disorder. Judging from the present experimental data, it was considered that red discoloration has a close relation to chlorosis appearing on the young plants of var. Tongil, because the two phenomena were derived from the nitrogen-fertilizing deficiency in leaves.

As will be illustrated later, there was an obvious difference in the occurrence of discoloration on leaves between var. Tongil and Punggwang, when an insufficient level of nitrogen was applied and when the nitrogen absorption of roots was limited by inhibited root respiration with metabolic inhibitors as 2,4-dinitrophenol and sodium sulfide.

Table 1 and Fig. 1 show that influence of nitrogen fertilizing on the difference of chlorosis(discoloration) in between var. Tongil and Punggwang. Tongil was observed to be more sensitive to nitrogen with discoloration than Punggwang with normal green leaves. At the level of 50mg/l ammonium sulfate, approximately 1cm discoloration from the tip of leaves to the below appeared in Punggwang, while more than 2 cm discoloration appeared on Tongil. No noticeable discoloration appeared on Punggwang at the level of 250 mg/1 ammonium sulfate; on the other hand, approxmiately 1 cm discoloration appeared on var. Tongil at the same level of ammonium sulfate. The observations thus made suggest that var. Tongil requires more nitrogen to perform a normal physiological function than Punggwang does. The discolored(chlorotic) leaves of Tongil were well recovered and started forming chlorophyll when an additive nitrogen is supplied. Seedlings of Punggwang at a very early stage of growth hardly became chlorotic even without supply of nitrogen, because necessary level of nitrogen originates from endosperm is sufficient to maintain leaves green for a while.

According to Kwack and Koo[2], the appearance of red discoloration on the paddy fields had a close relation to nitrogen levels applied regardless of minor elements present or absent such as Mn, B, Al, and Si, etc., therefore, and the more severe red discoloration appeared when more nitrogen absorption became limited.

The optical density between var. Tongil and Punggwang was determined in order to express the results in figures(Table 4, Fig. 4). The optical density of Punggwang was 0.47 at the level of 50 mg/l ammonium sulfate, and that of Tongil was 0.15 at the same level. This shows clearly that chlorophyll level is significantly higher in Punggwang leaves than Tongil. DNP of a low level plays a part in inhibiting the function of root respiration[4], and the effect of DNP of 100 mg/l was striking on inducing discolored leaves of Tongil, no such abnormality was noted in the presence of sufficient supply of nitrogen(Table 2, Fig. 2). Higher levels of DNP (50 and 100 mg/l) almost completely prevented leaves of all varieties from forming chlorophyll and resulted in showing differences in the length of leaves(Table 2). The optical density in Tongil at 10 mg/l DNP treatment was 0.25 and that in Punggwang was 0.45(Table 5, Fig. 5). Likewise the effect of sodium sulfide on the appearance of leaf discoloration was more outstanding in var. Tongil than in Punggwang(Table 3, Fig. 3). Approximately 0.6 cm of the discoration appeared on Punggwang at the level of 200 mg/l sodium sulfide, while 3 cm discoloration on Tongil at the same level of sodium sulfide. Thus there was a conspicuous difference in optical density on spectrophotometry between var. Tongil and Punggwang as treated with sodium sulfide(Table 6, Fig. 6).

The fact that chlorophyll content lowers with reduction of nitrogen supply for var. Tongil, whereas that is not the case for var. Punggwang, the red discoloration of Tongil suggests a gradual failure of forming chlorophyll within the leaves when insufficient nitrogen was applied. It seems to be therefore feasible to realize that difference in the nitrogen requirement and response between the two varieties is strictly genetical, and Tongil becomes manifested with more carotenoid(orange tinge) color on leaves than Punggwang as chlorophyll fades away with shortage of nitrogen, as was previously discussed[1,2,3].

The effect of respiratory inhibitors, DNP and Na_2S, on limiting root absorption of nitrogen and the formation of chlorophyll in the leaves, even in the presence of sufficient supply of nitrogen, was more pronounced in the young seedlings of var. Tongil than those of var. Punggwang, indicating that this phenomenon is also attributed to a varietal characteristic peculiar to Tongil. Judging from this, it can be said that the occurrence of discoloration on leaves of Tongil seedlings, although it does not appear to be red like that of the mature plants, is brought about by insufficient nitrogen application for the growth and by the various factors which may either directly or indirectly limit the nitrogen absorption from roots and hinder accumulation of nitrogen in leaves, such as poor drainage, low temperature, and any toxic substance for root respiartion, etc.(even if sufficient nitrogen was applied).

Summary

The present experiments were carried out to find out and confirm the cause of red discoloration on leaves of var. Tongil by artifical induction of it with certain metabolic inhibitors. Rice var. Tongil and Punggwang were compared each other in this study as to their responses to nitrogen feeding with two chemical respiratory inhibitors, DNP(2,4-dinirtophenol) and Na_2S(sodium sulifide), under the conditions of ample nitrogen supply. Rice seedlings were grown in beakers containing ammonium sulfate as nitrogen source. Conclusions can be briefed from the present studies as follows;

1. Rice var. Tongil(IR 667) showed more sensitive response to nitrogen feeding than Punggwang(a native Japonica type), indicating that Tongil requires more nitrogen for the maintenance of normal growth than Punggwang. The discoloration, thus, appears to result in chlorophyll degradation with shortage of nitrogen supply in the leaf tissues.

2. Appearance of leaf discoloration was more striking on var. Tongil than Punggwang with both DNP and Na_2S introduced as metabolic inhibitors in the cultural media, since these inhibitors apparently restricted the absorption of nitrogen through roots.

3. It seems to be very likely that the red discoloration of rice var. Tongil is brought about primarily by insufficient supply of nitrogen for the growth and by various factors which may limit the absorption of nitrogen through roots, such as low temperature, toxic gases or substances around the root zone, and poor drainage, etc., even if sufficient level of nitrogen is provided.

Table 1. Influences of nitrogen application on the leaf length and discoloration in rice var. Tongil and Punggwang(beaker grown).

$(NH_4)_2SO_4$ (mg/l)	Rice var. Punggwang**		Rice var. Tongil**	
	Leaf length (cm)	Discoloration (ea)	Leaf length (cm)	Discoloration (ea)
0	6.3	2.0	7.0	3.0
50	6.2	1.0	8.0	2.0
100	7.3	1.0	9.2	2.0
150	6.2	1.0	9.2	1.0
200	7.2	1.0	9.0	1.0
250	6.3	0	8.4	1.0

** Measured from the tip of leaves to the below.

Table 2. Response of rice var. Punggwang and Tongil to 2,4-dinitrophenol(beaker grown), 2,4-dinitrophenol is designated as DNP.

2,4-dinitrophenol* (mg/l)	Rice var. Punggwang**		Rice var. Tongil	
	Leaf length (cm)	Discoloration (ea)	Leaf length (cm)	Discoloration (ea)
0	10.4	0	11.1	0.5
2	9.2	1.0	11.9	2.9
10	5.1	1.0	7.8	4.0
50	3.9	3.9	6.6	6.6
100	3.8	3.9	6.0	6.0

* 0...250mg/l $(NH_4)_2SO_4$ alone, 2...250mg/l $(NH_4)_2SO_4$ plus 2mg/l DNP, 10...250mg/l $(NH_4)_2SO_4$ plus 10mg/l DNP, 50...250mg/l $(NH_4)_2SO_4$ plus 50mg/l DNP, 100...250mg/l $(NH_4)_2SO_4$ plus 100mg/l DNP

** Measured from the tip of leaves to the below

Table 3. Comparison of the leaf length and extent of the discoloration in rice var. Punggwang and Tongil when treated with Na_2S(beaker grown).

Na_2S* (mg/l)	Rice var. Tongil**		Rice var. Punggwang**	
	Leaf length (cm)	Discoloration (ea)	Leaf length (cm)	Discoloration (ea)
0	8.0	0.5	7.9	0
10	8.0	1.0	8.0	0.5
100	7.0	2.0	7.0	0.5
200	6.0	3.0	7.0	0.6
500	6.0	3.0	6.0	1.0

* 0...250mg/l $(NH_4)_2SO_4$ alone, 10...250mg/l $(NH_4)_2SO_4$ plus 10mg/l Na_2S, 100...250mg/l $(NH_4)_2SO_4$ plus 100mg/l Na_2S, 200...250mg/l $(NH_4)_2SO_4$ plus 200mg/l Na_2S, 500...250mg/l $(NH_4)_2SO_4$ plus 500mg/l Na_2S.

** Measured from the tip of leaves to the below.

Table 4. Comparison of the optical density between rice var. Punggwang and Tongil leaves when treated with nitrogen.

$(NH_4)_2SO_4$ (mg/l)	Optical density*	
	Rice var. Punggwang (rate)	Rice var. Tongil (rate)
0	0.40	0.15
50	0.47	0.15
100	0.60	0.47
150	0.60	0.60
200	0.70	0.75
250	0.95	0.85

* Optical density was checked at 5800 Å with 14mg dried leaves of the two rice varieties extracted with alcohol(95%) for 30 min.

Table 5. Difference in the optical density between rice var. Punggwang and Tongil leaves when treated with 2, 4-dinitrophenol.

2, 4-dinitro-phenol (mg/l)	Optical density*	
	Rice var. Punggwang** (rate)	Rice var. Tongil** (rate)
0	0.85	0.70
2	0.95	0.60
10	0.45	0.26
50	0.15	0.10
100	0	0.05

* Checked at 5800 Å for chlorophyll.

** Two varieties were grown in 250mg/l $(NH_4)_2SO_4$.

Table 6. Comparison of the optical density between rice var. Punggwang and Tongil leaves when treated with Na_2S.

Na_2S (mg/l)	Optical density*	
	Rice var. Punggwang** (rate)	Rice var. Tongil** (rate)
0	0.95	0.91
10	0.85	0.80
50	0.75	0.47
100	0.75	0.40
200	0.70	0.20
500	0.60	0.15

* Checked at 5800 Å for chlorophyll.

** Two varieties were grown in 250mg/l $(NH_4)_2SO_4$.

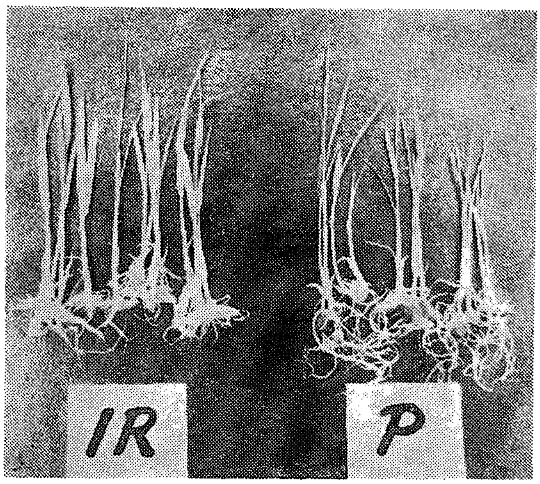

Fig. 1. Difference in extent of the characteristic chlorosis shown on between var. Tongil (IR) and Punggwang (P) leaves when fed with 50mg/l ammonium sulfate.

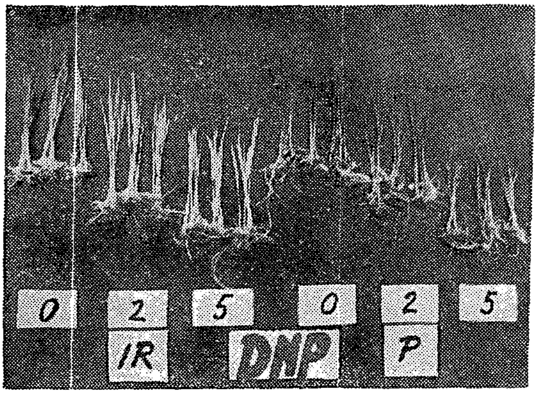

Fig. 2. Difference in extent of the characteristic chlorosis on between var. Tongil (IR) and Punggwang (P) leaves with varied levels of 2, 4-dinitrophenol(DNP) (0, 2 and 5mg/l) with 250mg/l ammonium sulfate.

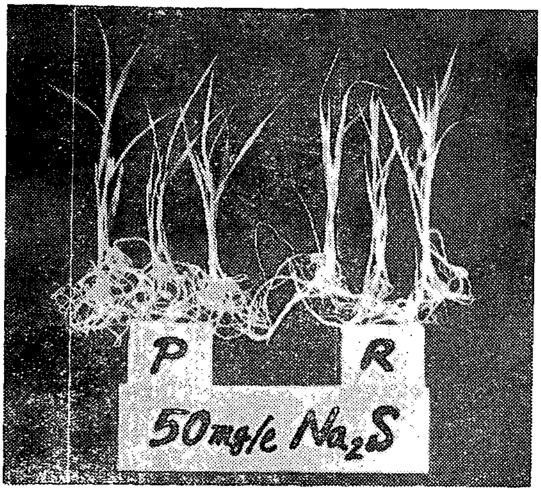

Fig. 3. Difference in extent of the characteristic chlorosis when treated with 50mg/l Na_2S between var. Punggwang(P) and Tongil(R) in the presence of 25mg/l ammonium sulfate.

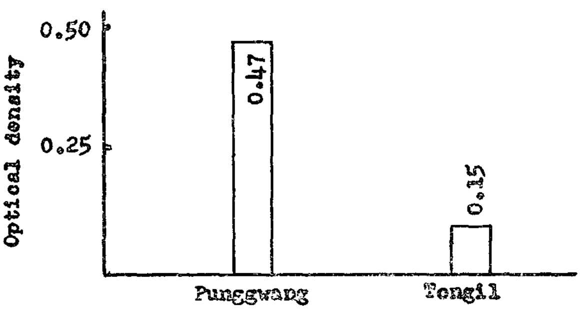

Fig. 4. Difference in the optical density at 5800 Å (chlorophyll band) between rice var. Punggwang and Tongil leaves when treated with 50mg/l ammonium sulfate.

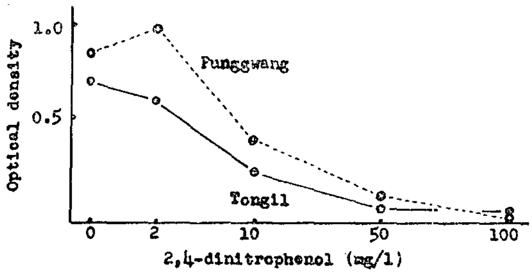

Fig. 5. Difference in the optical density at 5800 Å (chlorophyll band) between rice var. Punggwang and Tongil leaves when treated with varied levels of 2, 4-dinitrophenol in the presence of 250mg/l ammonium sulfate.

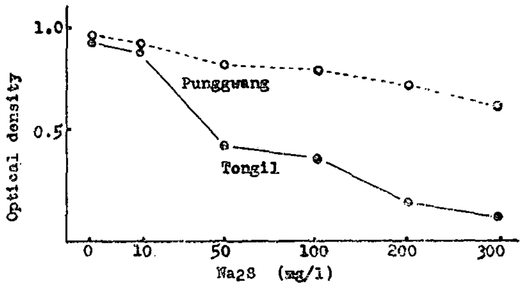

Fig. 6. Comparison of the optical density at 5800 Å (chlorophyll band) between rice var. Punggwang and Tongil leaves when treated with varied levels of sodium sulfide in the presence of 250 mg/l ammonium sulfate.

References

1. Ahn, S.D. 1972. Influence of increased leaf-nitrogen level on red discoloration of rice var. IR667. M.S. thesis, Agronomy, Korea University.
2. 郭炳華, 具英書. 1971. 水稻 IR 667 의 赤枯現象에 미치는 窒素의 영향에 關한 硏究, 植物學會誌 14: 141-149.
3. Kwack, B.H. 1974. Studies on red discoloration in rice var. Tongil(IR 667). Res. Rept., ORD, Minist. Agr. Fish., Vol. 17: in print.
4. 三井進午. 1971. 植物の榮養と肥料の硏究(三井博士論文選集). pp. 3-8, 30-33, 43-46, 養賢堂(東京).
5. 한국일보. 1971. 8月 29日, #7024.
6. 한국일보. 1971. 9月 2日, #7028.
7. 原田二郎. 1970. 水稻の葉の老化防止に及ぼすジ

ベレリン及カイネチンの影響, 農及園 45:1571-1572.

8. 農村振興廳. 1973. 벼新品種 統一이 우리나라 農政에 미친 영향.
9. 農村振興廳. 1972. 統一벼의 短點改善試驗(植物環境硏究所 1972年 事業評價資料), 新品種統一栽培法確立試驗(作物試驗場 1972年 事業評價資料).
10. 農林部. 1971. "통일"벼 재배법 〈녹색革命 성공의 길〉.
11. Lehman, W.F., M.L. Peterson, C.R. Adair, L.L. Davis and R.W. Haubrich. 1970. Rice introductions tested for use in California. Calif. Agr. 24(6):4-6.

摘　　要

本試驗은 통일(IR 667) 벼의 赤枯現象의 原因이 根本的으로는 질소 肥料의 不足과 土壤內에 存在하는 뿌리의 呼吸抑制條件(例를 들면 低溫, 排水不良 등)이 질소 吸收를 沮害시키는 結果라고 보고 赤枯現象의 本質을 더욱 究明하기 爲하여 遂行한 것이다.

水稻品種 통일(水原 214號)과 풍광을 질소 肥料와 呼吸阻止劑에 對한 反應으로 나타나는 黃化 또는 赤枯葉을 比較하였는데 代謝 또는 呼吸阻止劑로서는 2,4-dinitrophenol과 황화소다, 질소 給源으로서는 황산안몬을 使用 했다. 本 試驗의 結果를 要約하면 다음과 같다.

1. 통일벼는 在來種인 풍광에 比해서 질소에 보다 敏感한 反應을 보였으며 이는 통일이 풍광에 比하여 多量의 질소를 要求하는 品種이며 질소의 缺乏時는 통일은 葉綠素形成이 沮害되어 풍광은 그렇지 않는데 所謂 赤枯現象을 일으켰다.

2. 特히 2,4-dinitrophenol과 황화소다와 같은 呼吸阻止劑가 뿌리의 질소吸收에 미치는 영향은 통일벼가 풍광 보다 顯著하였으며 통일의 境遇 赤枯現象의 前提인 甚한 黃化現象이 나타났다.

3. 위의 두 가지 事實을 미루워 보아 통일벼의 잎에 나타나는 赤枯現象은 질소肥料의 供給 또는 吸收不足과 葉內의 질소成分不足으로 일어나는 品種自體의 特性에서 오는 現象이라고 말할 수 있다.