Plant Science, 44 (1986) 7—11
Elsevier Scientific Publishers Ireland Ltd.

IS MALE-STERILITY IN PLANTS RELATED TO LACK OF CYANIDE-RESISTANT RESPIRATION IN TISSUES?

MARY E. MUSGRAVE, JANIS ANTONOVICS and JAMES N. SIEDOW*

Department of Botany, Duke University, Durham, NC 27706 (U.S.A.)

(Received November 13th, 1985)
(Revision received January 15th, 1986)
(Accepted January 17th, 1986)

Cyanide-resistant (alternative) respiration by tissues of 7 male-sterile lines from 4 species (*Glycine max* (L.), *Helenium amarum* (Raf.) H. Rock., *Plantago lanceolata* L. and *Zea mays* L.) was compared with that of fertile tissues. Six of the 7 male-sterile lines lacked alternative respiration in the tissue assayed (leaf or root), while the corresponding fertile tissue displayed a typical alternative pathway equivalent to about 20% of the uninhibited respiratory rate. The exception to this pattern was the male-sterile maize line cytoplasmic male sterile (cms)-T, which had an alternative pathway equivalent to that of the fertile line. Mitochondria isolated from male-sterile cms-C maize were found to have an alternative pathway, but the capacity of this pathway was only one-half to two-thirds as large as that found in mitochondria from the male-fertile N line. Thus while no alternative pathway could be detected in intact cms-C tissue, it was found in the isolated mitochondria, suggesting that the pathway is somehow suppressed in the intact tissue. Since cytokinins are known to both inhibit the alternative pathway and affect floral development, they represent potential candidates for the suppressing agent involved. The lack of alternative respiration in the male-sterile lines might also serve to explain the commonly observed higher productivity and vigor of male-sterile plants compared to fertile ones.

Key words: alternative respiration; cyanide-resistant respiration; male-sterility

Introduction

Although the cyanide-resistant, alternative respiration pathway was first described some 60 years ago [1], its physiological significance remains unknown. While its participation during thermogenesis in some aroids has been documented [2] and a role has been postulated in facilitating respiration by various cyanogenic plant organs [3], an explanation of its general significance has been lacking. Lambers [4] suggested that plants may use this pathway to respire carbohydrate which cannot be readily stored or used in growth or reproduction. This idea was an extension of Palmer's earlier proposal that the presence of a non-phosphorylating respiratory pathway in plants, which have a ready supply of ATP due to photosynthesis, would permit the continued processing of carbon skeletons under conditions of high energy charge [5]. Since the alternative pathway bypasses two energy conservation sites on the cytochrome chain, electron flow through the alternative pathway will be energetically wasteful in comparison to activity through the main respiratory chain. Some have therefore viewed the alternative pathway as an inefficient evolutionary relic retained in some groups and lost in others [6].

Following up on observations by Miller [7] and Dizengremel et al. [8] that high concentrations (1 mM) of cytokinins inhibit cyanide-resistant respiration in isolated mitochondria, we recently showed a disengagement of the alternative pathway during the course of the response to 10^{-5} M benzyl-

*To whom correspondence should be sent.
Abbreviations: BSA, bovine serum albumin; cms, cytoplasmic male-sterile; PVPP, polyvinylpolypyrrolidone; SHAM, salicylhydroxamic acid.

0168-9452/86/$03.50

adenine in five separate cytokinin bioassays [9]. Furthermore, cytokinin-like responses were obtained when inhibitors of the alternative pathway (such as salicylhydroxamic acid (SHAM) or propyl gallate) were applied in several of these bioassays [10], strengthening the idea that cyanide-resistant respiration may be involved in some plant responses to cytokinins.

A number of reports have linked endogenous levels of cytokinins to male-sterility in plants [11–13]. Moreover, Champault [14] had demonstrated that exogenous application of cytokinins to apices of *Mercurialis annua* in culture can change the sex of the flowers produced. In view of our evidence regarding cytokinin effects on the alternative pathway, we surveyed a number of male-sterile plants to see whether any relationship could be found between the presence and/or extent of the alternative pathway and male-sterility.

Materials and methods

Plant materials

G. max (L.) Merr. Field grown plants of line N69-2774 segregating for the male-sterile character were generously provided by Drs. Joe Burton and R.F. Wilson, N.C. State University, Raleigh, NC. Plants homozygous for the male-sterile locus (msms) were male-steriles and bore relatively few pods compared with the heterozygous male-fertile plants (Msms) [19].

H. amarum (Raf.) H. Rock. Plants were located on a railroad right-of-way near the Duke campus. One individual was found to bear male-sterile flowers which completely lacked anthers although the pistils were normal in development. For respiration measurements, 10 young, field-collected leaves were used per sample.

P. lanceolata L. Male-sterile individuals collected from several sites in the Durham area were classified according to the system of van Damme and van Delden [20] as MS1 (vestigial anthers) or MS2 (anthers totally lacking). These four plants and two hermaphrodites were transplanted to pots in the Duke University lath house to allow several weeks' recovery and growth prior to respiratory determinations.

Z. mays L. seeds with the B73 nuclear background carrying normal, cms-C, cms-S or cms-T cytoplasms were provided by Dr. C.S. Levings III, N.C. State University, Raleigh, NC.

Methods

For respiration studies, maize seeds were surface sterilized for 10 min with a 10% (v/v) chlorox solution and then coated with Captan prior to germination in the dark (29°C). Roots from 4-day-old seedlings grown in germination paper were cut into 1-cm pieces and used in tissue respiration measurements as described below. Washed mitochondria were prepared from similar seedlings using minor modifications of previous procedures [21]. Root tissue (40 g) was ground with mortar, pestle and sand using grinding buffer containing 4% (w/v) polyvinylpolypyrrolidone (PVPP). Following isolation by differential centrifugation and washing, mitochondria were assayed using a Clark-type electrode and a reaction mixture containing 10 mM succinate, 150 μM ADP, and 0.1% (w/v) bovine serum albumin (BSA), in the standard mitochondrial reaction buffer [21].

Tissue respiration was determined according to Musgrave and Siedow [9]. Leaf material was dipped briefly in a 1% (v/v) ethanol solution prior to assay in a 2.0-ml cuvette. Potassium phosphate buffer (0.1 M) (pH 6.3) was circulated around the tissue and respiration rates were measured polarographically at 25°C using a Clark-type electrode. Potassium cyanide (2 mM) followed by 1 mM SHAM was used to determine the capacity of the cyanide-resistant pathway as a percentage of the total rate.

Results

Table I summarizes the results obtained for tissue respiration in the four species

Table I. Tissue respiration rates in fertile and male-sterile plants of four species. Mean total respiration, V_T, is expressed as nmol O_2 min^{-1} (g fresh wt.)$^{-1}$. Alternative respiration, expressed as a percentage of the total rate, was determined in the presence of 2 mM KCN by addition of 1 mM SHAM. Values are means of at least 3 experiments with standard errors in parentheses.

Species	Tissue	Line	V_T	% V_{alt}
G. max	Root	N	257 (27)	18 (4)
		MS1	240 (22)	0
H. amarum	Leaf	N	175 (11)	20 (1)
		MS	189 (14)	0
P. lanceolata	Leaf	N	420 (62)	17 (2)
		MS1	377 (38)	0
		MS2	365 (45)	0
	Root	N	323 (58)	21 (1)
		MS2	404 (44)	0
Z. mays	Root	N	450 (27)	24 (2)
		cms-C	421 (45)	0
		cms-S	407 (23)	0
		cms-T	532 (86)	28 (3)

surveyed. In general, alternative pathway activity was absent in the male-sterile tissue while fertile tissues showed a cyanide-resistant, SHAM-sensitive respiration equivalent to about 20% of the total, uninhibited rate, V_T. Three cytoplasmic male-sterile races of *Zea mays*, all having an isonuclear background, were compared with the fertile line (N). The male-sterile lines cms-C and cms-S lacked the alternative pathway in seedling root tissue, however race cms-T showed alternative respiration comparable to the fertile line (about 20% V_T).

Comparing mitochondrial respiration in the isonuclear cms-C and N maize lines, the cms-C mitochondria were found to have an alternative pathway capacity which did not appear in the respiration measurements in vivo. Table II summarizes the data for a representative experiment. Typically, the cms-C mitochondria had a lower alternative pathway capacity than the N mitochondria by 10—25% of the total uninhibited rate.

Table II. Respiration by mitochondria isolated from 4-day-old corn root tissue. Total (state 3) rates are expressed as nmol O_2 min^{-1} (mg protein)$^{-1}$ with succinate (10 mM) as the substrate. The capacity of the alternative pathway (V_{alt}) is expressed as a percentage of the state 3 rate and was determined by the inhibition of respiration following addition of 1 mM SHAM in the presence of 0.25 mM KCN. Values are means of at least 3 experiments with standard errors in parentheses.

Cytoplasm	State 3 respiration	% V_{alt}
Normal	60 (4)	63 (6)
cms-C	87 (8)	37 (2)

Discussion

The striking feature of these data is the general lack of the alternative pathway in male-sterile tissue. This pattern appears across a rather wide taxonomic range (Compositae, Gramineae, Plantaginaceae, Leguminosae). The one exception, the cms-T maize line is puzzling but may perhaps be understood when respiration is studied in the mature plant. Of the four species studied, all except the maize were in the reproductive phase at the time of the respiration measurements. Kuiper was reported to have looked at the respiration pattern in root tissue of *P. lanceolata* [18] and found a lower alternative pathway activity in roots from male-sterile *Plantago* (1.5 mg O_2 h^{-1} (g dry wt.)$^{-1}$) than in roots from fertile ones (3.0 mg O_2 h^{-1} (g dry wt.)$^{-1}$) while total respiration rates were comparable. When root respiration was measured in the male-sterile *Plantago* in the present study, close agreement was found with the leaf respiration data (Table I); the male-sterile plants totally lacked alternative respiration in the tissues. Why cyanide-resistant respiration in roots of *Plantago* was only lowered in male-steriles compared to fertiles in Kuiper's study [18] but totally absent in our plants is unknown, however a number of different types of male-steriles have been described in *Plantago* [20] so it is

quite possible that different types of male-steriles were used in the two studies.

Male-sterility in plants is known to be produced by a variety of nuclear/cytoplasmic interactions [22]. In natural populations such as *Plantago* and *Helenium* in this study, it is unclear whether male-sterile and -fertile plants differ in cytoplasmic or nuclear restorer genes or both. In some cases such as *Glycine*, different nuclear genes on a uniform cytoplasmic background determine the male-sterile condition, while in *Zea*, male-sterility is a cytoplasmic trait and our comparisons were made on an isonuclear background. It is interesting to note that although the male-sterility is determined by cytoplasmic differences in *Zea* and nuclear genetic differences in *Glycine*, the same pattern of lack of cyanide-resistant respiration in the male-steriles is observed.

Given the striking coincidence of male-sterility and lack of alternative pathway, two hypotheses are suggested. The first involves the possibility that the lack of alternative respiration is responsible for male-sterility. However, the occurrence of alternative respiration in cms-T maize tissue makes the relationship between lack of the pathway and male-sterility in the other lines appear more coincidental than causative. Further, mitochondria isolated from cms-C tissue (which lacks alternative respiration) display the pathway in vitro, suggesting a possible inhibition of the alternative pathway mediated by some component of the cytoplasm. A second hypothesis for the coincidence between male-sterility and lack of alternative pathway in tissues would postulate that some regulatory substance is influencing both the alternative pathway and the sex of the flowers.

Flavell [23] was among the first to propose that a plant growth regulator was involved in male-sterility. Durand and co-workers have provided many lines of evidence implicating cytokinins as the regulatory substance hypothesized by Flavell, at least in *Mercurialis* spp. where high endogenous levels of cytokinins as well as exogenous application of synthetic cytokinins evoke male-sterility [12—14]. Exogenous application of synthetic cytokinins have also been observed to greatly reduce or eliminate the production of male flowers in cultured spinach apices [15] and hemp plants [16,17]. Miller [7] and Dizengremel et al. [8] found that high concentrations of cytokinins inhibit alternative respiration in isolated mitochondria, and in a previous report, we found that lower concentrations (10^{-5} M) of benzyladenine evoked a disengagement of the alternative pathway in tissue respiration during responses by five separate bioassays to this cytokinin [9]. The present work coupled with these earlier observations on the inhibitory effects of cytokinins on the alternative pathway is consistent with the idea that higher cytokinin levels in male-sterile vs. fertile tissues could give rise to both the male-sterile condition and the apparent lack of cyanide-resistant respiration in whole tissues.

Another interesting feature of the observation that male-sterile tissues lack the alternative pathway has to do with the greater vigor of male-sterile plants as compared to their fertile counterparts. Van Damme and van Delden [24] and Primack [25] showed that in *P. lanceolata*, male-steriles exceeded the hermaphrodite plants significantly in such parameters as adult plant survival rate, seed production and weight per seed. A similar trend has been observed in the male-sterile maize races (C.S. Levings III, pers. commun.). Wilson et al. [19] have noted that male-sterile soybeans have higher leaf and root carbohydrate reserves even prior to podfill than the fertile counterparts do.

Various explantions for the differences in vigor between male-sterile and fertile plants have been proposed including inbreeding depression of hermaphrodites due to their recent history of selfing [26] or pleiotropic effects of one or more of the sex determining genes [27]. Darwin [28] first proposed that this pleiotropic effect might be explained in terms of 'compensation' whereby male-

steriles would invest carbon resources saved by not forming stamens in seed production.

Our present data may suggest another explanation. Since the alternative pathway bypasses two energy conservation sites on the cytochrome pathway, it has been hypothesized [4] that use of the alternative pathway renders plants less energetically efficient than use of the cytochrome pathway. Kuiper [29] has provided some evidence that indices of vigor might be inversely related to the amount of alternative pathway present in a plant. In two lines of *P. major* he found that a line with slow root growth (and few seeds per capsule) had a higher alternative respiration rate than a second line with faster root growth (and many seeds per capsule). Recently, we compared the growth of pea hybrids differing only in the presence or absence of the alternative pathway. Plants lacking the pathway outperformed those with the pathway in terms of seed production, total dry weight and specific leaf weight (unpublished results). We therefore suggest that the observed greater vigor of male-steriles may be a consequence of a lack of alternative pathway activity in the tissues.

In conclusion, although this study deals with only 4 species and 7 lines of male-steriles, it points out the potential importance of alternative respiration in relation to male-sterility. Further experiments are currently underway.

Acknowledgements

This work was supported by NIH grant GM 26095 to J.N.S. M.E.M. acknowledges support from a James B. Duke Graduate Fellowship and the N.I.H. Cell and Molecular Biology training grant (T32-GM 07184).

References

1 L. Genevois, Rev. Gen. Bot., 41 (1929) 252.
2 B.J.D. Meeuse, Annu. Rev. Plant Physiol., 26 (1975) 117.
3 H.C. Passam, Plant Sci. Lett., 7 (1976) 211.
4 H. Lambers, Plant Cell Environ., 3 (1980) 293.
5 J.M. Palmer, Annu. Rev. Plant Physiol., 27 (1976) 113.
6 H. Lyr and T. Schewe, Acta Biol. Med. Ger. 34 (1975) 1631.
7 C.O. Miller, Proc. Natl. Acad. Sci. U.S.A., 77 (1980) 4731.
8 P. Dizengremel, M. Chauveau and J. Roussaux, Plant Physiol., 70 (1982) 585.
9 M.E. Musgrave and J.N. Siedow, Physiol. Plant., 64 (1985) 161.
10 M.E. Musgrave and J.N. Siedow, Plant Physiol., 78 (1985) S-110.
11 H. Ahokas, Proc. Natl. Acad. Sci. U.S.A., 79 (1982) 7605.
12 G. Kahlem, A. Champault, J.P. Louis, M. Bazin, A. Chabin, M. Delaigue, B. Dauphin, R. Durand and B. Durand, Physiol. Veg., 13 (1975) 763.
13 J.P. Louis, and B. Durand, Mol. Gen. Genet., 165 (1978) 309.
14 A. Champault, Bull. Soc. Bot. Fr., 120 (1973) 87.
15 L. Culafic and M. Neskovic, Physiol. Plant., 48 (1980) 588.
16 M.K. Chailakhyan and V.N. Khryanin, Planta, 138 (1978) 185.
17 M.K. Chailakhyan, and V.N. Khryanin, Planta, 138 (1978) 181.
18 H. Lambers, Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms, in: R. Douce and D.A. Day (Eds.), Encyclopedia of Plant Physiology, Vol. 18, Springer-Verlag, Berlin, 1985, p. 459.
19 R.F. Wilson, J.W. Burton, J.A. Buck and C.A. Brim, Plant Physiol., 61 (1978) 838.
20 J.M.M. van Damme and W. van Delden, Heredity, 49 (1982) 303.
21 S.J. Stegink and J.N. Siedow, Plant Physiol., 80 (1986) 196.
22 M. Hanson and M.F. Conde, Int. Rev. Cytol., 94 (1985) 214.
23 R. Flavell, Plant Sci. Lett., 3 (1974) 259.
24 J.M.M. van Damme, and W. van Delden, Evolution, 38 (1984) 1326.
25 R.B. Primack, New Phytol., 81 (1978) 449.
26 B. Charlesworth, and D. Charlesworth, Am. Nat., 112 (1978) 975.
27 S. Wright, Evolution, 34 (1980) 825.
28 C. Darwin, Different Forms of Flowers on Plants of the Same Species, Murray, London, 1877.
29 D. Kuiper, Physiol. Plant., 57 (1983) 222.