Plant & Cell Physiol. 22(1): 65–71 (1891)

Necessity of a Balance between CN-Sensitive and CN-Resistant Respirations for Germination of Cocklebur Seeds

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When applied singly, KCN or NaN_3, as inhibitors of the cytochrome respiration path, and benzohydroxamic acid or *n*-propyl gallate, as inhibitors of the alternative respiration path, were less effective and ineffective, respectively, in inducing germination of secondarily dormant, upper seeds of *Xanthium pennsylvanicum* Wallr. When applied in combination, however, these chemicals were very effective, producing much higher gemination. Thus, we concluded that an appropriate balance between the cytochrome and alternative path fluxes is required to induce the germination of secondarily dormant cocklebur seeds.

Key words: Alterntive respiration — Azide — Benzohydroxamic acid — Cocklebur seed — Cyanide — Germination — Propyl gallate — Secondary dormancy.

A variety of seeds possess both CN-sensitive and CN-resistant respiration pathways, the latter of which is necessary to ensure germination (Burgvillo and Nicolás 1977, Siedow and Girvin 1980, Yentur and Leopold 1976, Yu et al. 1979). Both pathways are known to be involved in the respiration of cocklebur seeds and to be indispensable for their germination because the presence of KCN or NaN_3, as conventional respiration inhibitors of the CN-sensitive cytochrome path, and that of BHAM or SHAM, as the inhibitor of the CN-resistant alternative path (Schonbaum et al. 1971), delayed germination (Esashi et al. 1979b, Esashi et al. in press b). Nevertheless, prior treatments with KCN or NaN_3 stimulate the germination of cocklebur seeds and overcome their secondary dormancy (Esashi et al. 1979a), although KCN and NaN_3 strongly inhibit O_2 uptake during an early extended period following KCN or NaN_3 treatment (Esashi et al. in press b). Thereafter, KCN or NaN_3 produces an increase in O_2 uptake, especially through the CN-resistant alternative path, which subsequently results in germination (Esashi et al. in press a). This phenomenon has been explained as the follows: KCN and NaN_3 promote seed germination by increasing the ratio of the alternative path flux to the cytochrome path flux by inhibiting the latter. This is based on the assumption that an appropriate balance between the two fluxes is necessary for seed germination (Esashi et al. in press a, b). Another possibility is that the germination-stimulating action of KCN and NaN_3 may be activated indirectly because respiration substrates are saved from consumption in the presence of KCN or NaN_3,

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Abbreviations: BHAM, benzohydroxamic acid; SHAM, salicylhydroxamic acid; PG, *n*-propyl gallate.

resulting in intensified respiration that leads to the stimulation of germination after their removal (Esashi et al. in press b). If so, the effect of KCN and NaN_3 should be enhanced as a result of the great saving of respiration substrates through inhibition of both the cytochrome and alternative pathways due to the simultaneous administration of both inhibitors. The present study was undertaken to determine which explanation is correct.

Materials and Methods

Secondarily dormant upper seeds of cocklebur (*Xanthium pennsylvanicum* Wallr.), which previously had been kept in a water substrate at 23°C for more than 3 months and which had nearly lost sensitivity to KCN and NaN_3 for germination, were used in most experiments. Seeds were placed in a 9-cm Petri dish containing 2 discs of filter paper and 10 ml of the test solutions. After treatment at 23°C for 24 hr, seeds were rinsed throughly with water then transferred to water for the germination test. In the experiment shown in Table 1, seed which had undergone imbibition for four weeks and which were at the early stage of secondary dormancy, but still responsive to C_2H_4 and KCN were used. We exposed them in a 125-ml flask, which contained 2 discs of filter paper and 4 ml of KCN solution. Ethylene gas was added by syringe in the necessary volumes, to the flask through its rubber stopper. Seeds were sown 60 to 75 to the dish and 20 to 23 to the flask in triplicates. Germination was recorded at intervals as the appearance of the radicle tip through the seed coat or the cracking the seed coat. Each experiment was repeated at least 2 or 3 times.

Results and Discussion

Secondarily dormant seeds were placed in contact with various concentrations of KCN with or without 20 or 30 mM BHAM for 24 hr prior to the transfer to water. Untreated seeds remained dormant. As in earlier studies (Esashi et al. 1979a, in press a), KCN alone had a very slight effect on germination. In contrast, combined applications of KCN and BHAM led to loss of secondary dormancy, thus inducing germination. Interestingly, BHAM promoted the induction of germination at 20 mM when combined with 10 or 15 mM KCN, whereas it caused maximal germination at 30 mM in combination with 25 mM KCN.

We have suggested that both the great capacity of the alternative path and a balance between the alternative and cytochrome paths are required for the germination of cocklebur seeds (Esashi et al. in press a, b). The stronger inhibition of the alternative respiration flux by BHAM that was required to induce maximal seed germination when inhibition of the flux via the cytochrome path was almost complete (Esashi et al. in press b), is additional support for the position that an appropriate balance is needed for the induction of seed germination. Our present results also indicate that the germination-stimulating action of KCN and NaN_3 does not result from the more saving of respiration substrates alone.

We recently have found that BHAM promotes C_2H_4 production in cocklebur cotyledons (Satoh and Esashi 1980). Ethylene, not cytokinins or gibberellins, is the crucial plant hormone that stimulates cocklebur seed germination (Esashi et al. 1977, 1978). Therefore, the position that BHAM promotes germination by

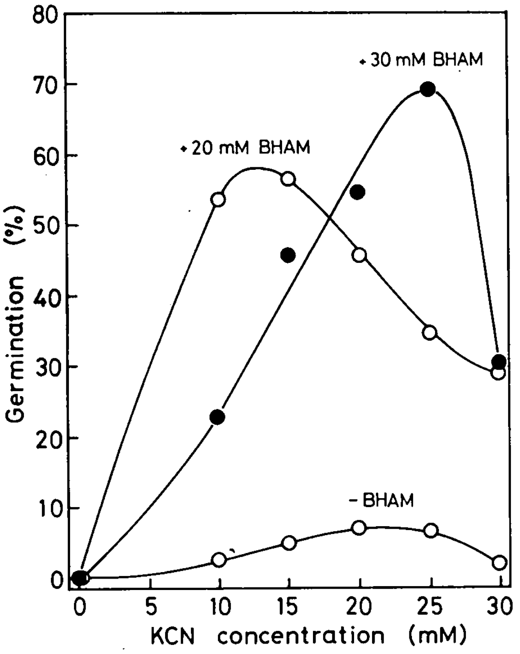

Fig. 1

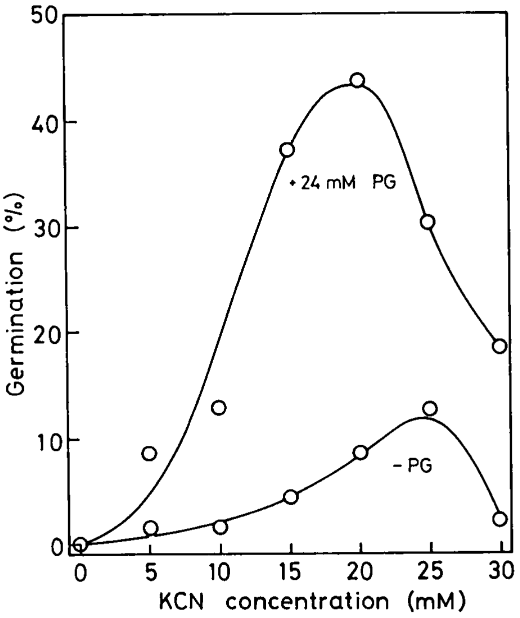

Fig. 2

Fig. 1 Stimulation of germination of secondarily dormant cocklebur seeds due to simultaneous applications of KCN and BHAM. Upper cocklebur seeds that had been soaked for 3 months were treated with KCN and BHAM in combination for 24 hr, then they were washed and transferred onto a water substrate. Germination was recorded 78 hr after transfer. l.s.d.(5%) = 9.5

Fig. 2 Stimulation of germination of secondarily dormant cocklebur seeds due to simultaneous applications of KCN and PG. Upper cocklebur seeds that had been soaked for 98 days were treated with KCN and PG in combination for 24 hr, then they were washed and transferred onto a water substrate. Germination was recorded 78 hr after transfer. l.s.d.(5%) = 8.9

keeping an appropriate balance between the fluxes of the two paths may be incorrect. The following 2 experiments were done to test the possibility that the C_2H_4 produced abundantly in the presence of BHAM may enhance the effectiveness of KCN.

First, a test was made to determine whether the effectiveness of KCN on germination is enhanced by the simultaneous application of C_2H_4 (Table 1). When applied singly, both KCN and C_2H_4 stimulated germination of seeds which were developing secondary dormancy. In combination, however, KCN and C_2H_4 antagonized each others action; the germination-stimulating effect of C_2H_4 was erased by the presence of KCN. This suggests that both the operation of the alternative path and that of the cytochrome path are necessary for C_2H_4-stimulated germination as well as for the normal germination (Esashi et al. in press b). Contrary to expectation, the promotive effect of KCN was reduced by an C_2H_4 application. Ethylene is known to raise the O_2 uptake, especially via the alternative path, in imbibed cocklebur seeds (Esashi et al. 1979b, in press a). Therefore, the increasing alternative flux due to C_2H_4 concomitant with the decreasing cytochrome flux caused by KCN seems to be an inadequate condition for germination. Both the excessive operation of the cytochrome path and that of the alternative path may be unfavorable for inducing the germination of cocklebur seeds.

Table 1 Interaction of KCN and C_2H_4 in the germination of cocklebur seeds

Concentration of KCN (mM)	Germination (%)		
	0	1 μl/ml C_2H_4	10 μl/ml C_2H_4
0 (H_2O)	0	57.9	82.3
10	51.5	40.7	7.4
20	64.0	33.9	2.8

Upper seeds that had been soaked for 4 weeks were treated with KCN and C_2H_4 in combination for 48 hr, then washed and transferred onto a water substrate. The percentage of germination was scored 80 hr after the start of treatments. l.s.d.(5%) = 9.1

The second experiment was performed with PG as the inhibitor of alternative respiration (Siedow and Girvin 1980) in place of BHAM. Results are shown in Fig. 2. Unlike BHAM, PG is known to inhibit C_2H_4 production (Baker et al. 1978). As in the results shown in Fig. 1, only combined treatments with KCN and PG effectively induced the germination of secondarily dormant cocklebur seeds. And the results shown in Fig. 2 demonstrate the importance of the balance between the alternative and cytochrome fluxes for inducing the germination of cocklebur seeds.

Additional evidence for this balance is seen in Table 2, in which NaN_3 and BHAM at different concentrations were applied simultaneously to seeds. Maximal germination took place when 1.5 mM NaN_3 was combined with 20 mM BHAM. Because the ratio of the alternative flux to the cytochrome flux in cocklebur seeds is considered to be very small in the secondary dormancy state (Esashi et al. 1979b, in press a), a higher ratio appropriate to the induction of germination must be achieved in the presence of lower concentrations of KCN or NaN_3. However, a single application of 5 mM KCN (Fig. 2) or 0.5 mM NaN_3 (Table 2), which could not completely inhibit O_2 uptake in this seed (Esashi et al. in press b), had little effect on germination. Probably both an appropriate balance between the alternative and cytochrome fluxes and the saving of respiration substrates are necessary for the germination-stimulating effects of simultaneous applications of KCN or NaN_3 and BHAM or PG.

Table 2 Stimulation of germination of secondarily dormant cocklebur seeds due to the simultaneous application of NaN_3 and BHAM

Concentration of NaN_3 (mM)	Germination (%)			
	0	20 mM BHAM	30 mM BHAM	40 mM BHAM
0 (H_2O)	0	0	0	0
0.5	1.4	3.1	1.6	2.8
1.0	16.7	55.4	47.6	38.3
1.5	22.8	65.4	60.0	45.4

Upper cocklebur seeds that had been soaked for 102 days were treated with NaN_3 and BHAM in combination for 24 hr, then washed and transferred onto a water substrate. The percentage of germination was scored 98 hr after transfer. l.s.d.(5%) = 8.8

The germination-stimulating effect of combined treatments of KCN and BHAM is produced only with simultaneous applications (Table 3). When given following KCN treatment, BHAM prevented KCN-induced germination as reported previously (Esashi et al. in press b), giving evidence that the engagement of the alternative path is necessary for the KCN response in cocklebur seeds. When given prior to KCN treatment, BHAM had no effect.

Normal germination of lower cocklebur seeds proceeds through sequential processes characterized by different O_2 requirements and inhibitor sensitivities: the anaerobic, the CN-sensitive, the CN-resistant and the CN-sensitive phases (Esashi et al. in press b, Katoh and Esashi 1975). The process requiring the operation of the alternative path is the limiting step of normal germination in cocklebur seeds. Secondary dormancy of upper cocklebur seeds is believed to develop because of the diminished capacity of the alternative path at ordinary temperatures (Esashi et al. 1979b). Further support for this is that secondary dormancy in the lower cocklebur seed, having a high germination potential because of the greater capacity of the alternative path (Esashi et al. in press b), is induced in the presence of the alternative respiration inhibitor (unpublished). In contrast, secondary dormancy could be terminated by treatment, such as an C_2H_4 application, especially in enriched O_2, which would increase both the flux via the alternative path and its ratio against the flux via the cytochrome path (Esashi et al. in press a). The data given in Table 3 suggest that both an appropriate ratio and a large capacity need not be present at the same time during the germination-limiting process for germination to be induced.

Taylorson and Hendricks (1973) reported that lettuce seeds, like cocklebur seeds, are capable of germinating in response to KCN in darkness. In contrast, Yu et al. (1979) observed the stimulatory action of SHAM at a low concentration on the germination of lettuce seed, but they did not satisfactorily explain the reason for this. This paradox may result from increased C_2H_4 production in contact with a low concentration of SHAM, since C_2H_4 is known to stimulate the germination of lettuce seeds (Abeles and Lonski 1969, Burdett and Vidaver 1971) and SHAM to enhance C_2H_4 production in seed tissues (Satoh and Esashi 1980). There is another possible explanation for this paradox. In cocklebur seeds, KCN

Table 3 Effects of separate and simultaneous applications of KCN and BHAM on the germination of secondarily dormant cocklebur seeds

Treatment		Germination (%)	
First 24 hr	Second 24 hr	20 mM BHAM	30 mM BHAM
H_2O	H_2O	0	0
H_2O	KCN	1.4	1.4
H_2O	BHAM	0	0
H_2O	KCN+BHAM	64.3	51.8
KCN	BHAM	0	0
BHAM	KCN	8.5	7.1

Upper cocklebur seeds that had been soaked for 106 days were treated with 10 mM KCN and 20 or 30 mM BHAM in combination as described in the Table, then they were washed and transferred onto a water substrate. Germination was recorded 73 hr after transfer. l.s.d.(5%)=7.3

was stimulatory when the cytochrome path was dominant, whereas BHAM was promotive when the flux via the alternative path was superior because of the presence of KCN or NaN_3. The experimental temperature adopted by Yu et al. (1979) differs from that used by Taylorson and Hendricks (1973), the former was 26°C whereas the latter was 33°C. If the balance between the alternative and cytochrome fluxes is strikingly different due to temperature, the their conflicting results can be explained. If the ratio of the alternative flux to the cytochrome flux is very high at high temperatures, lettuce seeds could germinate in response to KCN. If the ratio is very low at low temperatures, SHAM could cause seeds to germinate. The difference in the balance between the alternative and cytochrome fluxes in cocklebur seeds differs due to temperature will be described elsewhere.

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(Received August 30, 1980; Accepted December 2, 1980)