

## REVIEW

### REGULATION OF NITRATE REDUCTASE ACTIVITY IN HIGHER PLANTS

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**Abstract**—Nitrate reductase is one of the most important enzymes in the assimilation of exogenous nitrate—the predominant form of nitrogen available to green plants growing in soil. Activity of this enzyme in plants gives a good estimate of the nitrogen status of the plant and is very often correlated with growth and yield. Although it is difficult to explain the physiological significance and the mechanism of effects of several factors on the enzyme activity, in some cases suitable postulates have been advanced. In general, the enzyme activity in a plant tissue is a balance between its relative rates of synthesis/degradation and activation/inactivation. Factors may affect the overall activity by interfering with either of these processes.

Most higher plants growing in soil obtain their nitrogen in the form of nitrate. In the pathway  $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+ \rightarrow$  amino acids, reduction of nitrate to nitrite by the enzyme nitrate reductase (NR, EC 1.6.6.1) is believed to be the rate-limiting step [1]. This is indicated by the fact that while nitrate may accumulate in the plant tissues, nitrite and ammonium do not [2, 3]. Significant positive correlations between nitrate reductase activity (NRA) and plant growth have been observed in *Zea* [4], *Populus* [5] and *Triticale* [6]. In several systems, enzyme activity is correlated with grain protein and nitrogen [7-14]. A positive correlation between NRA and reduced nitrogen at low level of nitrogen supply has been observed in many graminaceous plants [15]. Even in diseased plants increase in NRA is closely correlated with the increase in ethanol-soluble and protein nitrogen of the host [16]. In *Hordeum vulgare* and *Zea mays*, supply of nitrate induces NRA and development of peroxisomes [17]. Since supply of ammonium also increases peroxisome number, it is concluded that induced NRA supplies reduced nitrogen for the synthesis of glycine and serine in the peroxisomes and therefore their number correlates with the NRA [17]. Thus, NRA may serve as an index of the nitrogen status of the plant. Although the literature on the reduction of nitrate to nitrite has been reviewed by Hageman and Hucklesby [18], Hewitt [19] and Hewitt *et al.* [20], they concentrated mostly on the physico-chemical nature of the enzyme. A review by Beevers and Hageman [1] had incorporated work on the effects of environmental factors on NRA but since then the

literature has grown enormously. In the present paper, a critical appraisal of the effects of different factors on NRA is presented.

#### SUBSTRATE INDUCTION

The presence of NRA has been demonstrated in almost all the tissues examined. Workers have demonstrated that the lack of activity in *Malus sylvestris* leaves [21] could be related to inadequate extraction and assay procedures [22, 23]. The enzyme is readily induced by the supply of nitrate in *H. vulgare* aleurone layers [24] and leaves [25, 26], embryos of *Agrostemma githago* [27], *Z. mays* tissues [28-31], *Brassica cauliflora* [32, 33], *Spirodela oligorrhiza* [34], *Lemna minor* [35], *Oryza sativa* seedlings [36, 37], *Raphanus sativus* [38], *Cucurbita pepo* cotyledons [39], *Pisum sativum* buds [40], *Crotalaria juncea* pollen [41] and many other systems. In cases where tissue nitrate does not correlate with NRA [42], it is possible that most of the nitrate is in a storage pool rather than in a metabolic pool. In a typical inducing system, the enzyme activity increases linearly after 0.5 hr of nitrate supply, reaching a maximum after 3-4 hr [30]. The initial lag period (0.5 hr) may represent the time taken for the uptake and translocation of nitrate and the expression of NR specific gene(s). By using labelled nitrate, it has been shown that nitrate-induced NRA is of a protein synthesized *de novo* [43]. This is supported by data from other investigations involving the use of protein synthesis inhibitors [44-47].

Table 1. Saturating concentration of nitrate for the induction of nitrate reductase activity

Plant species	Approximate concentration of NO <sub>3</sub> (mM)	Reference
<i>Agrostemma githago</i> embryo	50	49
<i>Gomphrena globosa</i>	4.0	50
<i>Gossypium hirsutum</i> leaves	100	51
<i>Hordeum distichum</i> roots	0.02	44
<i>H. vulgare</i>		
—aleurone layers	100	24
—leaves	3.0	50
<i>Lemna minor</i>	0.5	35
<i>Lolium perenne</i>	0.5	52
<i>Pisum sativum</i>		
—leaves	4.0	53
—shoot tips	40	54
<i>Prunus</i> leaves	10	23
<i>Raphanus sativus</i>		
—cotyledons	10	55
—seedlings	10	56
<i>Triticum aestivum</i> leaves	3.0	50
<i>Zea mays</i>		
—endosperm	100	57
—scutellum	50	57, 58
—root tips	50	58
	10	59
—mature root sections	50	58
—entire roots	25	60
—leaves	3.0	50

Nitrate is essential for the maintenance of NRA. When it is withdrawn from the medium, the enzyme from *Z. mays* and *Nicotiana tabacum* cells loses activity with a half life of ca 4 hr [29, 43]. The rate of loss of activity, however, may also depend upon the tissue. For example, the enzyme has a faster rate of decay ( $t_{0.5} = 2$  hr) in mature root sections than that in the root tips ( $t_{0.5} = 3$  hr) [46]. Since neither nitrate nor NADH protect the decay of purified NR from *H. vulgare* leaves [48] it may be postulated that the protecting effect of nitrate on crude enzyme preparations is indirect.

Saturation-type kinetics are observed with various concentrations of nitrate. The concentration at which maximum activity is induced varies from species to species (Table 1). This may reflect the differences in the rates of uptake of nitrate among species and/or their capacity to mobilise nitrate to the metabolic pool. The concentration of nitrate at which half maximum velocity of uptake ( $K_m$ ) is observed differs from species to species [61, 62].

#### AMMONIUM AND AMINO ACIDS

The effect of ammonium and amino acids as potential end products of nitrate assimilation, on NRA, has been examined by several workers. Plants with ammonium salts generally have lower NRA than those grown with nitrate [37, 63]. When ammonium is supplied with nitrate, the substrate induction of enzyme is substantially inhibited in *L. minor* [35, 64, 65] and roots of *Hordeum distichum* [44] and *Gossypium hirsutum* [66]. In *Vaccinium*, ammonium completely inhibits NRA at pH 4 but increases it at pH 6 [67].

Ammonium grown *Wolffia arhiza* plants have very low levels of NRA but the enzyme is induced when the plants are transferred to a medium containing ammonium nitrate [68]. Many other workers have failed to demonstrate inhibition of NRA by ammonium [58, 69–71]. This may reflect the differences in the regulation of NRA in different tissues. A closer examination of the methods used shows that differences in response to the ammonium may also be attributed, at least in some cases, to the composition of the media.

Ammonium may inhibit NRA either by limiting the uptake of nitrate [72] or by interfering with its synthesis. Alternatively it may inhibit the activity of the enzyme either by active degradation or by physical modulation of the enzyme molecule. The evidence for the latter possibility has been recorded in algae, where it has been shown that inactivation of NRA by ammonium is reversible [73]. That enzyme inactivation may be a probable mechanism of action of ammonium in higher plants also is indicated by the fact that addition of ammonium to nitrate-induced plants results in a rapid loss of enzyme activity [74]. It has also been suggested that ammonium induces the synthesis of a regulatory protein specific for nitrate reductase [35].

Ammonium increases enzyme induction in cultured *Rosa* [75] and *Triticum aestivum* cells [76] and in *Phaseolus aureus* seedlings [77, 78]. Since ammonium has no effect on *in vitro* enzyme level [75], it is concluded that ammonium increases NRA by promoting its synthesis. It is possible that the tissues, where an increase in enzyme induction is observed, are deficient in amino acids and that supply of ammonium to

those cells increases the pool of amino acids available for the synthesis of enzyme.

Inhibition of NR by a mixture of amino acids (casein hydrolysate) has been demonstrated in *N. tabacum* cells [79] and *Z. mays* [71]. While each of the individual amino acid tested inhibits induction of NR in *L. minor* [35], only a few have any effect on enzyme activity in many other cases [34, 79–83], and the inhibitory effect of one amino acid is reversed by others [79, 83]. In cultured *Glycine max* cells [76], alanine or putrescine increases NRA in the presence of 2,4-dichlorophenoxy acetic acid while glutamine and glutamate have no effect. Several amino acid analogues such as DL-2-fluorophenyl alanine, DL-7-azatryptophan, L-canavanine, L-ethionine and thio-proline inhibit induction of NRA in *A. githago* [84]. However, the corresponding amino acids have no effect on the enzyme activity.

As is the case with ammonium, amino acids may inhibit NRA by inhibiting any of the steps from uptake of nitrate to the synthesis and activity of enzyme. Inhibition of NRA in maize root tips by the amino acid analogue canavanine is observed to be on the synthesis of the enzyme [85]. This inhibition is reversible by arginine and it has been suggested that the effect of canavanine is related to its incorporation into an inactive protein [86]. Incorporation of canavanine in NR protein has yet to be shown, however. The physiological significance and the mechanism of induction of enzyme by some amino acids is not understood at present. While interpreting these observations, it must be noted that the amino acids supplied from outside and those generated *in situ* are channelled to different pools [87].

Inhibition of NRA by hydroxylamine, another possible product of nitrate reduction has also been reported [88].

#### INORGANIC SALTS AND IONS

The effect of various inorganic salts and ions on NRA has been studied. One of the most widely studied salts is molybdate. Since Mo is a component of the NR molecule [19, 89], NRA in Mo-deficient plants is low but increases rapidly when Mo is supplied exogenously [32, 90–93]. Further, the level of Mo is correlated positively with NRA in *Citrus* leaves [94]. Tungsten (W) is a competitive inhibitor of Mo and when it is incorporated in the enzyme molecule the activity is very low [19, 95]. Thus an exogenous supply of W inhibits induction of NRA in *N. tabacum* cells [96, 97], embryos of *A. githago* [84] and *H. vulgare* seedlings [98]. However, the existing level of NR is not affected by the supply of W [99].

The effect of NaCl on NRA is rather varied. While it induces activity in the cotyledons of *Phaseolus aconitifolium* [100] and *Z. mays* shoots [101], it inhibits NRA in *Atriplex confertifolia* at a concentration of 50 meq/l. [102]. Several monovalent cations, such as  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Rb}^+$ , have no effect on *in vitro* NRA in *Spinacea oleracea* but *in vivo* activity is strongly induced by  $\text{Rb}^+$  [103]. In *T. aestivum* seedlings, induction of NRA was 2 to 3 times higher in the presence of  $\text{KNO}_3$  than that with  $\text{NaNO}_3$  [104]. The deficiency of the divalent cation  $\text{Ca}^{2+}$  decreases NRA in *C. pepo* plants [105].

The effect of sulphur (S) levels on NRA has also been studied. Since NR is a —SH containing molecule [1], deficiency of S decreases NRA in *Z. mays* [106]. However, when S is not limiting, its supply has no effect on NRA in *Phleum pratense* and *Panicum virgatum* [107]. Brown and Jones [108] compared the effects of Fe supply on calcicolous and calcifugous *Lycopersicon esculentum* plants. Fe deficiency decreased NRA in the roots and shoots of calcicoles while it had no effect in calcifuges.

#### SUGARS AND ORGANIC ACIDS

Glucose as a source of energy for the induced synthesis of NRA has been tested by some investigators. It increases induction of NRA in carbohydrate depleted green *H. vulgare* leaves in light and in dark [26]. On the other hand, it has no effect on either induction or loss of NRA in the normal leaves of *Perilla frutescens* [109]. Addition of glucose does not prevent the loss of NRA under non-inducing conditions ( $-\text{NO}_3^-$ ) [59]. It is proposed that glucose affects NRA by determining the distribution of nitrate in roots.

Many compounds of carbohydrate metabolism also affect NRA. Citrate induces NRA in *Cucumis sativus* cotyledons which is maximum at pH 3 [110]. Since the induction by citrate is inhibited by cycloheximide it may be concluded that the induction involves *de novo* synthesis of the enzyme. Knypl and Ferguson [110] have also reported that NRA is induced by Hepes buffer, acetate, ascorbate, aspartate, glutamate, oxalate, succinate and tartrate at pH 3 but not at pH 6.

#### ANTIBIOTICS AND METABOLIC INHIBITORS

Since substrate induction of NR involves *de novo* synthesis, the process is inhibited by actinomycin D in *H. distichum* roots [44] and by cycloheximide in *L. minor* [35], *O. sativa* seedlings [36] and *H. vulgare* [24]. Cycloheximide delays the loss of NRA when barley leaves are transferred from light to darkness [111]. On the other hand, soaking seeds in actinomycin D has little effect on NRA after 6–50 hr of imbibition but the enzyme in immature embryos is activated by the antibiotic [112]. Actinomycin D also stimulates NR induction in *G. hirsutum* seedlings when the germination is preceded by a 3 hr soaking in actinomycin D [112]. This might have resulted from the inhibition of the synthesis of a negative effector of NR.

Inhibition of NR induction by puromycin is also reported, although it is required at relatively higher concentration [44]. Chloramphenicol stimulates NRA in *O. sativa* [113, 114], *Z. mays* seedlings [115] and in *C. sativus* cotyledons [116]. On the other hand, it inhibits enzyme induction in *Phaseolus vulgaris* [117]. In *Z. mays*, when seeds are soaked in chloramphenicol for 24 hr and then germinated in the presence of nitrate for 3 days, the enzyme activity is increased by 27 and 44% in the roots and shoots, respectively [118]. Floating leaves on chloramphenicol, however, inhibits the induction of enzyme [119]. In *A. githago*, chloramphenicol has no effect on benzyladenine-induced enzyme but it inhibits nitrate-induced enzyme activity [84].

Chloramphenicol is a specific inhibitor of protein synthesis on 70 S ribosomes [120] while NR is reported to be synthesized in the cytoplasm on 80 S ribosomes [121]. The cytoplasmic location of the enzyme has also been demonstrated by other workers [122]. In cases where chloramphenicol inhibits induction of NRA it is possible that a part of the NR molecule is synthesized in the cell organelles containing 70 S ribosomes. Even the procedure of Ritenour *et al.* [121], adopted to demonstrate the cytoplasmic location, does not exclude the possibility that NR is located on the external membranes of the chloroplast. In cases, where chloramphenicol has stimulatory action, it may be postulated that it might be inhibiting a NR-specific inhibitor. It will be rather interesting to determine if the NR-specific inhibitor is synthesized in the cell organelles sensitive to the chloramphenicol.

Several metabolic inhibitors are also known to inhibit NRA. Inhibition of activity by cyanide has been reported in *H. distichum* [44], *T. aestivum* [123], *Z. mays* [70], *S. oleracea* [124] and *Glycine max* [125]. The fact that inhibition of enzyme is reversible [124] and that thiosulphate and kidney rhodanase restore the activity of the inhibited enzyme [123] suggest that binding between cyanide and NR is loose. Inhibitors such as sodium azide [125], phosphon D, CCC, B-nine and Amo-1618 [116] and heavy metals such as Pb and Cd [126] also inhibit NRA.

#### GROWTH REGULATORS

While gibberellins have no effect on the induction of NR in *H. vulgare* aleurone layers [127], GA and GA in combination with a cytokinin increase NRA in leaves [128]. Gibberellins and cytokinins applied simultaneously can substitute light for the maintenance of NRA in *N. tabacum* leaves [129]. Indole acetic acid also increases NRA and it has been established that the effect of growth substances on the enzyme is not due to stimulation of general protein synthesis [128]. Induction of NRA by cytokinins [49, 116, 130, 131] involves *de novo* protein synthesis [132] and therefore it is inhibited by actinomycin D and cycloheximide [133]. In *Trigonella foenum-graecum* seedlings, the enzyme activity is synergistically induced by nitrate and kinetin [134]. However, the mode of induction of NR by cytokinins is different from that of nitrate. While benzyladenine loses its effectiveness as an inducer after 24 hr of seed germination, the effect of nitrate is not time limited [135].

The growth regulator, abscisic acid, has no effect on enzyme activity in germinating *G. hirsutum* seeds [112] but it stimulates NRA in the cotyledons of *P. aconitifolium* [100]. Soaking seeds in ascorbic acid and salicylic acid simultaneously also increases *in vivo* NRA in *Z. mays* leaves [136].

The effect of various phenyl propanoids and their derivatives on the induction of NR in *Z. mays* seedlings has been examined by Schrader and Hageman [70]. Of the compounds tested, only transcinnamic acid and coumarin inhibit the induction of enzyme. The activity of NR in *L. esculentum* roots is unaffected by EDTA [137].

Certain nucleotides are known to affect the enzyme activity. The *in vitro* NRA is inhibited by ADP while ATP and AMP have no effect [137]. Inhibition of

NRA by ADP is reported for *O. sativa* seedlings [138] and *S. oleracea* [139] also. In *S. oleracea*, the inhibition changes from competitive to non-competitive in the presence of thiol compounds, such as glutathione, mercaptoethanol, thioglycollate and cysteine [139] or is completely reversed by glutathione [140]. This may suggest the involvement of the —SH group in the regulation of enzyme activity by the nucleotides.

Interaction among nitrate, NADH, ferrocytochrome *c* and cyanide in the regulation of different components of NRA has been studied in algae as well as in higher plants. NR is inactivated synergistically by NADH and ferrocytochrome *c* in the absence of nitrate. When nitrate is included, however, protection against inactivation is afforded involving ping pong (bi bi) kinetics [141, 142]. Perhaps these inactivators convert the enzyme to a lower  $V_{max}$  value state. Further, the presence of NADH improves the inhibitory effect of cyanide on the enzyme [143, 144]. It has been postulated that NADH causes over-reduction of Mo of the enzyme to a valency state less than the normal (6+) value, resulting in the increased binding of the cyanide to the enzyme [19]. Nitrate either protects the inactivating site for NADH on the enzyme by masking, or it suppresses the modulation of enzyme molecule to a state with lower  $V_{max}$  value [142].

#### HERBICIDES AND FUNGICIDES

Herbicides such as triazines, urea, diazines, bipyridyliums, phenols and benzonitriles inhibit NRA in *L. minor* but dinitroanilines and amides (excluding propanil) have no effect on the activity [145]. Ametryn inhibits enzyme activity in *T. aestivum* [146]. Inhibition of NRA by chlorate in *L. esculentum* plants is proportional to the concentration of the herbicide [147]. On the other hand, simazine increases NRA in *Z. mays* linearly up to 24  $\mu$ M [148]. Increase in NRA in *H. vulgare* and *Z. mays* seedlings also occurs with atrazine [149].

The fungicide chlorothalonil increases NRA by 20–30% when the leaves are treated with the fungicide 2 weeks before harvest. Inclusion of chlorothalonil in the extraction medium, however, inhibits NRA by 50% [150].

#### SEEDLING AGE AND DIURNAL RHYTHMS

Seedling age affects both the steady state and inducible levels of enzyme. The steady state level of the enzyme is normally low in the tissues of young seedlings, reaches a maximum in mature tissues and then declines again in the senescent ones [32, 40, 69, 151–157]. In *Carica papaya* fruits [158], in leaves of *H. vulgare* and *T. aestivum* [159] and in *Ipomoea batatas* [160], the activity is higher in the younger tissues and declines with age. In the flag leaf of *T. aestivum*, enzyme level is highest during anthesis and declines with leaf senescence [161]. In contrast to these observations, Murty and Sirohi [162] have reported that NRA in *T. aestivum* is high at 20 days, low at 50 days and again high at around 80 days of plant growth. This trend is apparently due to heterogeneity in the sample.

The inducible level of enzyme is also influenced by seedling age. In *Z. mays*, the enzyme is induced to a higher level in detached leaves from 6-day-old seedlings than those from 9-day-old seedlings [163]. Lower steady state level of enzyme in young tissues of the seedlings appears to be due to the inhibitory effect of organic nitrogen flowing from reserve proteins. In *P. vulgaris* seedlings, when cotyledons (the source of organic nitrogen) are removed, the enzyme activity in the young leaves is the highest [153]. As the capacity to induce NR in the leaves depends upon their efficiency to synthesize proteins [164], the lower NRA in overmature and senescent leaves, as observed in *Perilla* [109] and *Pisum* [165], might have been due to reduction or loss of protein-synthesizing capacity.

Like many other enzymes, a rhythm in NRA has also been reported. Diurnal variation with a peak at about mid-day has been observed in *Z. mays* [151, 166], *L. perenne* [52], *G. max* [167] and cotyledons of *G. hirsutum* [168]. Circadian rhythms in *G. max* and *Z. mays* have also been reported [169]. The enzyme shows periodicity when plants are kept in dark or in light [170, 171]. The periodicity in NRA appears to be correlated with nitrate and nitrite levels in *Chenopodium rubrum* seedlings [172]. In *Capsicum annum* leaves, 3 peaks of NRA have been reported. Peak I is observed soon after the end of photoperiod [173]; peak II is observed within 1 hr of start of photoperiod and peak III, the major peak, is observed 6 hr after light on [174]. In continuous light only two of these peaks are observed [175]. Since periodicity also exists in excised leaves, it is suggested that a leaf-based mechanism operates in the periodicity. In the duckweed *Wolffia microscopica*, cycloheximide inhibits increase in enzyme activity and it has been suggested that the development of an enzyme peak is due to *de novo* synthesis [176].

#### TEMPERATURE

Variation in temperature during seedling growth affects enzyme level in plants. In *Z. mays*, temperatures above 20° increase *in vivo* NRA, the most effective temperatures being 45° which causes a 3- to 4-fold increase in the activity [177]. Higher temperatures (above a certain optimum) inhibit NRA [29, 178–182]. However, the magnitude of inactivation by higher temperature varies according to species. For example, in a study of the effect of temperature (10–40°) on NRA in a cool season grass (*Agrostis palustris*) and a warm season grass (*Cynodon dactylon*), Kauffman *et al.* [183] noted 20° as the optimum temperature for both species. But the enzyme activity in *Agrostis* is greatly reduced when the seedlings are raised to 35° while it is little affected in *Cynodon* at that temperature. Inactivation of NRA at temperatures lower than optimum is not as drastic as at higher temperatures. In fact in *H. vulgare*, lower temperature protects enzyme activity to some extent against its natural dark inactivation [180].

Temperature modifies the effect of seedling age on enzyme activity. In *G. max* seedlings [184], NRA increases concurrently with leaf development and then decreases as leaf maturation progresses at 25 and 32.5°. At 40°, however, there is no initial increase and the activity decreases gradually as the leaf develops and matures.

#### LIGHT

Light is essential for the substrate induction of NR [28, 185, 186], although Beevers *et al.* [55] and Travis and Key [164] demonstrated the induction of NR in young *Z. mays* seedlings in the dark. In almost all cases studied, the level of NRA in the parts of light-grown plants is higher than those of dark-grown [56, 164, 187–189]. When dark-grown etiolated seedlings are transferred to light, the enzyme is induced progressively in the presence of nitrate [53, 170, 190, 191]. Similarly when light-grown seedlings are transferred to dark, the enzyme activity decreases [186, 192] with a half life of ca 22 hr [170]. In *G. max* leaves, the dark inactivation of NR is faster at 40° and slowest at 20° [193].

Besides its duration, the intensity of light also influences NRA. In *H. vulgare*, NRA is greater in plants grown at higher irradiance than at lower [194]. Increasing irradiance from 6 to 45 klx increases NRA in *G. max* [167] and from 6 to 40 klx in *P. sativum*, *T. aestivum*, *H. vulgare*, *Z. mays*, *Gomphrena globosa* and *C. pepo* [50].

Light-mediated induction of NRA seems to involve phytochrome. This assumption is based upon observations that the inducing effects are red/far red reversible [39] and that induction by red light is insensitive to cycloheximide [191]. Further, the protein synthesizing capacity of 80 S ribosomes (which are supposed to be the site of NR synthesis) is enhanced by a 5 min red light treatment and the effect is completely reversed by a brief far red treatment [195]. By examining both chlorophyllous and non-chlorophyllous tissues, it has been concluded that modulation of NRA by light is not through photosynthetic photoreceptors, but that phytochrome is involved in the photocontrol of NRA [196].

Several hypotheses have been proposed regarding the mechanism of induction-activation of NRA by light: (1) Initially it was proposed that light may increase NRA by increasing nitrate uptake [39, 55, 98, 197]. This proposal, however, may be applicable only to those cases where nitrate supply is really limiting. (2) From studies involving green tissues, it has been concluded that photosynthesis is involved in induction either directly [109, 198] or by increasing cytoplasmic protein synthesis [185]. However, the presence of NR in the dark-grown tissues [55, 164] and increase in its activity by light in non-chlorophyllous tissues, such as endosperm and scutellum [199], do not support this proposal. Further, DCMU at a concentration required to inhibit the photosystem II activity does not inhibit the induction of NR by light [200]. (3) Light may increase NRA by inducing protein synthesis. This hypothesis is based upon observations that light induces synthesis of mRNA [201, 202]. This hypothesis seems to be viable when we assume that under inducing conditions, i.e. when nitrate supply is adequate, light induces synthesis of NR molecules more than other proteins and hence there is an increase in the specific activity of the enzyme. However, the observation that induction of NR by light is insensitive to cycloheximide [191] does not support this hypothesis. (4) Recently Jolly and Tolbert [203] have demonstrated that NR levels in light and dark are regulated by relative activities of specific inhibitors and activators. They have proposed that the inhibitor is

formed in the dark and is reversibly inactivated by light. That an inhibitor does really exist, has been demonstrated in *H. vulgare* [186], *Z. mays* [204, 205], *O. sativa* seedlings [206, 207] and in cultured cells of *O. sativa*, *T. vulgare*, *G. max*, *N. tabacum* and *Ruta graveolens* [208].

Wright and Murphy [209] have examined the effect of UV irradiation on NRA. UV irradiation of cultured *N. tabacum* cells inhibits production of NR; subsequent illumination with white light allows limited synthesis of NR.

#### WATER STRESS

Decrease in water potential below  $-4$  to  $-2$  bars causes a decrease in NRA in *H. vulgare* [210], *Z. mays* [211, 212] and *G. hirsutum* [168]. Adverse effects of water stress have also been reported in other systems [177, 213–216].

Water stress may decrease NRA by inhibiting either nitrate uptake or protein synthesis. In an intact plant, reduced transpirational pull during water stress may cause a decline in nitrate flux into the tissues. However, the differences in nitrate concentration in wheat seedlings grown at different irrigation regimes is small and insignificant [214]. On the other hand, water stress leads to a decline in polyribosome levels in *Z. mays* [217] and both polyribosomal level and NRA recover after rewatering [211, 213].

#### GASEOUS ENVIRONMENT

Induction of NR in relation to  $\text{CO}_2$  supply has been studied in *Perilla* [109] and *Zea* leaves [218, 219]. Increasing the concentration of  $\text{CO}_2$  from 100 to 800  $\mu\text{l/l}$ . increases NRA in *Z. mays* leaves. But the plants pretreated with  $\text{CO}_2$  in the dark and maintained in an atmosphere of 100  $\mu\text{l/l}$ .  $\text{CO}_2$  in the light have almost twice the NRA of plants exposed to 600  $\mu\text{l/l}$ .  $\text{CO}_2$  after 5 hr of illumination [219]. The *in vitro* NRA is substrate inducible in the absence of  $\text{CO}_2$  but actual assimilation of nitrate is inhibited [220]. The enzyme activity in plant parts exposed to an atmosphere of nitrogen is normally higher than those exposed to a normal atmosphere [185]. High NRA is detectable in the leaves of barley plants grown in low  $\text{O}_2$  conditions, although the enzyme is not specific for NADH, unlike the normal assimilatory enzyme developed under aerobic conditions [221]. However, other characteristics of the enzyme are similar to the normal assimilatory enzyme. In many flood-tolerant species, when anaerobiosis is created by flooding, the enzyme activity is increased [222, 223]. In *H. vulgare* roots NRA increases in a low  $\text{O}_2$  environment [224]; however, in the same system, with a completely anaerobic atmosphere the reduction and assimilation of nitrate stopped or occurred at very slow rate [225].

A wind or wind plus sand at a speed of 13.4 m/sec for 40 min inhibited NRA in *G. max* seedlings [226].

Air pollutants are also known to affect NRA. In young *G. max* leaves, the enzyme is inhibited by 0.25 and 0.5  $\mu\text{l/l}$ . ozone, while in mature leaves only the higher concentration is inhibitory [227]. Further, the air pollutant does not seem to cause any permanent

effect since activity is recovered in most cases when the ozone is withdrawn. In another investigation a ozone-resistant cultivar of *G. max* (hood), NRA was not affected by a 2 hr exposure to 0.5  $\mu\text{l/l}$ . ozone while it was significantly inhibited in a sensitive variety (dare) [228]. Since the supply of exogenous sucrose eliminates inhibition of NRA by ozone, it has been suggested that the air pollutant inhibits NRA by interfering with carbohydrate metabolism.

#### CONCLUSION

Although the effects of various environmental and nutritional factors on NRA are well documented in various systems, the exact mechanisms at molecular level are understood only in a few cases. It is apparent, however, that the enzyme is sensitive to various factors and that it has a rapid turnover rate [29, 31, 43]. The factors may affect the synthesis and/or activity of the enzyme either directly or by changing the physico-chemical environment of the cell and the cell organelles. The occurrence of a NR-specific inhibitor and its sensitivity to various factors in many systems [186, 204–208] indicates that it is possibly a critical molecule in the regulation of NR and that some of the factors regulate enzyme activity through this molecule. The isolation of a NR-deficient mutant of haploid *N. tabacum* cells [229] and production of a diploid hybrid containing normal level of NRA, by fusing protoplasts of two lines of such mutants [230] are important recent contributions in this field. The information from these techniques may lead us to understand the mechanism of NR regulation at the molecular level.

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#### REFERENCES

1. Beevers, L. and Hageman, R. H. (1969) *Annu. Rev. Plant Physiol.* **20**, 495.
2. Spencer, D. (1958) *Handb. Pflanzenphysiol.* **8**, 201.
3. Hageman, R. H., Leng, E. R. and Dudley, J. W. (1967) *Adv. Agron.* **19**, 45.
4. Zieserl, J. F., Ravenback, W. L. and Hageman, R. H. (1963) *Crop Sci.* **3**, 27.
5. Dykstra, G. F. (1974) *Plant Physiol.* **53**, 632.
6. Singh, B., Sapra, V. T. and Patil, J. A. (1976) *Euphytica* **25**, 193.
7. Croy, L. I. and Hageman, R. H. (1970) *Crop Sci.* **10**, 280.
8. Deckard, E. L., Lambert, R. J. and Hageman, R. H. (1973) *Crop Sci.* **13**, 343.
9. Eilrich, G. L. and Hageman, R. H. (1973) *Crop Sci.* **13**, 59.
10. Hernandez, H. H., Walsh, D. E. and Baur, A. (1974) *Cereal Chem.* **51**, 530.
11. Dalling, M. J., Hallorn, G. M. and Wilson, J. H. (1975) *Aust. J. Agric. Res.* **26**, 1.
12. Johnson, C. B., Whittington, W. J. and Blackwood, G. C. (1976) *Nature* **262**, 133.
13. Deckard, E. L., Lucken, K. A., Joppa, L. R. and Hammond, J. J. (1977) *Crop Sci.* **17**, 293.
14. Asthana, J. S. and Srivastava, H. S. (1980) *Proc. Natl. Acad. Sci. (India)* (in press).

15. Austin, R. B., Rossi, L. and Blackwell, R. D. (1978) *Ann. Botany* **42**, 429.
16. Mukerjee, M., Srivastava, H. S. and Bhargava, K. S. (1979) *Indian J. Exp. Biol.* **17**, 116.
17. Fair, P. (1978) *Ann. Botany* **42**, 101.
18. Hageman, R. H. and Hucklesby, D. P. (1971) *Methods Enzymol.* **23**, 491.
19. Hewitt, E. J. (1975) *Annu. Rev. Plant Physiol.* **26**, 73.
20. Hewitt, E. J., Hucklesby, D. P. and Notton, B. A. (1976) in *Plant Biochemistry*. (Bonner, J. and Varner, J. E. eds.). Academic Press, New York.
21. Sanderson, G. W. and Cocking, E. C. (1964) *Plant Physiol.* **39**, 416.
22. Klepper, L. A. and Hageman, R. H. (1969) *Plant Physiol.* **44**, 110.
23. Leece, D. H., Dilley, D. R. and Kentworthy, A. L. (1972) *Plant Physiol.* **49**, 725.
24. Ferrari, T. E. and Varner, J. E. (1969) *Plant Physiol.* **44**, 85.
25. Bejerano, N. R. and Lips, H. S. (1973) *New Phytol.* **12**, 253.
26. Aslam, M., Oaks, A. and Huffaker, R. C. (1976) *Plant Physiol.* **58**, 588.
27. Fukuyama, D. M. and Kende, H. (1974) *Plant Physiol.* **54**, 821.
28. Hageman, R. H. and Flesher, D. (1960) *Plant Physiol.* **35**, 700.
29. Schrader, L. E., Ritenour, G. L., Eilrich, G. L. and Hageman, R. H. (1968) *Plant Physiol.* **43**, 930.
30. Stevens, D. L. and Oaks, A. (1973) *Can. J. Botany* **51**, 1255.
31. Aslam, M. and Oaks, A. (1976) *Plant Physiol.* **57**, 572.
32. Afridi, M. M. R. K. and Hewitt, E. J. (1964) *J. Exp. Botany* **15**, 251.
33. Afridi, M. M. R. K. and Hewitt, E. J. (1965) *J. Exp. Botany* **16**, 628.
34. Ferguson, A. R. (1969) *Planta* **84**, 353.
35. Stewart, G. R. (1972) *J. Exp. Botany* **23**, 171.
36. Shen, T. C. (1969) *Plant Physiol.* **44**, 1650.
37. Marwah, R. S. and Juliano, B. O. (1976) *Plant Physiol.* **57**, 923.
38. Stulen, I., Kockbosma, T. and Koster, A. (1973) *Acta Bot. Neer.* **22**, 581.
39. Jones, R. W. and Sheard, R. W. (1975) *Plant Physiol.* **55**, 954.
40. Amindari, S., Dirr, M. A. and Splittstoesser, W. E. (1978) *Plant Cell Physiol.* **19**, 299.
41. Kapor, A., Malick, C. P. and Dhawan, A. K. (1978) *Plant Cell Physiol.* **19**, 685.
42. Shaner, D. L. and Boyer, J. S. (1976) *Plant Physiol.* **58**, 499.
43. Zielke, H. R. and Filner, P. (1971) *J. Biol. Chem.* **246**, 1772.
44. Smith, F. W. and Thompson, J. F. (1971) *Plant Physiol.* **48**, 219.
45. Stewart, G. R. (1968) *Phytochemistry* **7**, 1139.
46. Oaks, A., Wallace, W. and Stevens, D. L. (1972) *Plant Physiol.* **50**, 649.
47. Jones, R. W., Abbott, A. J., Hewitt, E. J., Best, G. R. and Watson, E. F. (1978) *Planta* **141**, 183.
48. Sherrard, J. H. and Dalling, M. J. (1979) *Plant Physiol.* **63**, 346.
49. Kende, H., Hahn, H. and Kays, S. (1971) *Plant Physiol.* **8**, 702.
50. Jones, R. W. and Sheard, R. W. (1977) *Plant Physiol.* **59**, 535.
51. Radin, J. W. (1973) *Plant Physiol.* **51**, 332.
52. Bowermann, A. and Goodman, P. J. (1971) *Ann. Botany* **35**, 353.
53. Jones, R. W. and Sheard, R. W. (1972) *Can. J. Botany* **51**, 27.
54. Shilhag, R. K., Guha-Mukherjee, S. and Sapory, S. K. (1979) *Physiol. Plant.* **45**, 281.
55. Beevers, L., Schrader, L. E., Flesher, D. and Hageman, R. H. (1965) *Plant Physiol.* **40**, 691.
56. Stulen, I., Zanginge, J. and Koster, A. (1973) *Acta Bot. Neer.* **22**, 589.
57. Srivastava, H. S., Asthana, J. S. and Jain, Abha (1979) *Physiol. Plant.* **47**, 119.
58. Wallace, W. (1973) *Plant Physiol.* **52**, 191.
59. Aslam, M. and Oaks, A. (1975) *Plant Physiol.* **56**, 634.
60. Neyera, C. C. and Hageman, R. H. (1975) *Plant Physiol.* **56**, 692.
61. Rao, K. P. and Rains, D. W. (1976) *Plant Physiol.* **57**, 55.
62. Clement, C. R., Hopper, M. J. and Jones, L. H. P. (1978) *J. Exp. Botany* **29**, 453.
63. Frith, G. J. T. (1972) *Plant Cell Physiol.* **13**, 1085.
64. Joy, K. W. (1969) *Plant Physiol.* **44**, 849.
65. Orebamjo, T. O. and Stewart, G. R. (1975) *Planta* **122**, 27.
66. Radin, J. W. (1975) *Plant Physiol.* **55**, 178.
67. Townsed, L. R. (1970) *Can. J. Plant Sci.* **50**, 603.
68. Swader, J. A. and Stocking, C. R. (1971) *Plant Physiol.* **47**, 189.
69. Ingle, J., Joy, K. W. and Hageman, R. H. (1966) *Biochem. J.* **100**, 577.
70. Schrader, L. E. and Hageman, R. H. (1967) *Plant Physiol.* **42**, 1750.
71. Oaks, A., Aslam, M. and Boesel, I. (1977) *Plant Physiol.* **59**, 391.
72. Ferguson, A. R. and Bollard, E. G. (1969) *Planta* **88**, 344.
73. Losada, M., Herrera, J., Maldonado, J. M. and Paneque, A. (1973) *Plant Sci. Letters* **1**, 31.
74. Orebamjo, T. O. and Stewart, G. R. (1975) *Planta* **122**, 37.
75. Mohanty, B. and Fletcher, J. S. (1976) *Plant Physiol.* **58**, 152.
76. Bayley, J. M., King, J. and Gamborg, O. L. (1972) *Planta* **105**, 25.
77. Higgins, T. J. V., Goodwin, P. B. and Carr, D. J. (1974) *Aust. J. Plant Physiol.* **1**, 1.
78. Mohanty, B. (1978) *Plant Physiol.* s-375.
79. Filner, P. (1966) *Biochim. Biophys. Acta* **118**, 299.
80. Hewitt, E. J. and Notton, B. A. (1967) *Phytochemistry* **6**, 1329.
81. Sahlka, J. (1974) *Biol. Plant.* **14**, 308.
82. Behrend, J. and Mateles, R. I. (1976) *Plant Physiol.* **56**, 584.
83. Radin, J. W. (1977) *Plant Physiol.* **60**, 467.
84. Dilworth, M. F. and Kende, H. (1974) *Plant Physiol.* **54**, 821.
85. Srivastava, H. S. (1975) *Phytochemistry* **14**, 1951.
86. Aslam, M., Oaks, A. and Boesel, I. (1978) *Plant Physiol.* **62**, 693.
87. Oaks, A. (1965) *Plant Physiol.* **40**, 149.
88. Jawali, N., Sainis, J. K. and Sane, P. V. (1978) *Phytochemistry* **17**, 1527.
89. Nicholas, D. J. D. and Nason, A. (1955) *Plant Physiol.* **30**, 135.

90. Hewitt, E. J. and Afridi, M. M. R. K. (1959) *Nature* **183**, 57.
91. Mulder, E. G., Boxma, R. and Vanveen, W. L. (1959) *Plant Soil* **10**, 355.
92. Randall, P. J. (1969) *Aust. J. Agric. Res.* **20**, 635.
93. Merkel, D., Witt, H. H. and Jungk, A. (1975) *Plant Soil* **42**, 131.
94. Shaked, A. and Bar-Akiva, A. (1967) *Phytochemistry* **6**, 347.
95. Notton, B. A. and Hewitt, E. J. (1971) *Biochem. Biophys. Res. Commun.* **44**, 702.
96. Heimer, Y. M. and Filner, P. (1971) *Biochim. Biophys. Acta* **230**, 362.
97. Heimer, Y. M., Wray, J. L. and Filner, P. (1969) *Plant Physiol.* **44**, 1197.
98. Rao, K. P. and Rains, D. W. (1976) *Plant Physiol.* **57**, 59.
99. Wray, J. L. and Filner, P. (1970) *Biochem. J.* **119**, 715.
100. Sankhla, N. and Huber, W. (1975) *Z. Pflanzenphysiol.* **75**, 392.
101. Alina, B. A. and Klyshev, L. A. (1975) *Izv. Akad. Nauk. Kaz. SSR Ser. Biol.* **13**, 28.
102. Kleinkopf, G. E., Wallace, A. and Cha, J. W. (1975) *Soil Sci.* **120**, 45.
103. Pflüger, R. and Wiedemann, R. (1977) *Z. Pflanzenphysiol.* **85**, 125.
104. Blevins, D. G., Barnett, N. M. and Frost, W. B. C. (1978) *Plant Physiol.* **62**, 784.
105. Prasil, I. (1975) *Biol. Plant.* **17**, 223.
106. Friedrich, J. W. and Schrader, L. E. (1978) *Plant Physiol.* **61**, 900.
107. Friedrich, J. W., Smith, D. and Schrader, L. E. (1976) *Can. J. Plant Sci.* **57**, 1151.
108. Brown, J. C. and Jones, W. E. (1976) *Physiol. Plant.* **38**, 273.
109. Kanangara, C. G. and Woolhouse, C. G. (1967) *New Phytol.* **66**, 553.
110. Knypl, J. S. and Ferguson, A. R. (1975) *Z. Pflanzenphysiol.* **74**, 434.
111. Travis, R. L., Jordon, W. R. and Huffaker, R. C. (1969) *Plant Physiol.* **44**, 1150.
112. Radin, J. W. and Trelease, R. N. (1976) *Plant Physiol.* **57**, 902.
113. Shen, T. C. (1972) *Planta* **108**, 21.
114. Shen, T. C. (1972) *Plant Physiol.* **49**, 546.
115. Schrader, L. E., Beevers, L. and Hageman, R. H. (1967) *Biochem. Biophys. Res. Commun.* **26**, 14.
116. Knypl, J. S. (1973) *Z. Pflanzenphysiol.* **70**, 1.
117. Sluiter-Scholten, C. M. Th. (1973) *Planta* **113**, 229.
118. Srivastava, H. S. (1976) *Indian J. Plant Physiol.* **19**, 53.
119. Tripathi, R. D., Srivastava, H. S. and Dixit, S. N. (1977) *Indian J. Exp. Biol.* **19**, 51.
120. Ellis, R. J. (1969) *Science* **163**, 477.
121. Ritenour, G. L., Joy, K. W., Bunning, J. and Hageman, R. H. (1967) *Plant Physiol.* **42**, 233.
122. Wallsgrove, R. M., Lea, P. J. and Mifflin, B. J. (1979) *Plant Physiol.* **63**, 232.
123. Tomati, U., Sermanni, G. G., Dupre, S. and Canella, C. (1976) *Phytochemistry* **15**, 597.
124. Notton, B. A. and Hewitt, E. J. (1971) *FEBS Letters* **18**, 19.
125. Jolly, S. O., Campbell, W. and Tolbert, N. E. (1976) *Arch. Biochem. Biophys.* **174**, 431.
126. Venkatramanna, S., Veeranjanyulu, K. and Ramdas, V. S. (1978) *Indian J. Exp. Biol.* **16**, 615.
127. Ferrari, T. E. and Varner, J. E. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **65**, 729.
128. Roth-Bejerano, N. and Lips, H. (1970) *New Phytol.* **69**, 165.
129. Lips, H. and Roth-Bejerano, N. (1969) *Science* **166**, 109.
130. Borris, H. (1967) *Math Naturwiss. Rieche* **16**, 629.
131. Kende, H. and Shen, T. C. (1972) *Biochim. Biophys. Acta* **286**, 118.
132. Hirshberg, K., Hubner, G. and Borris, H. (1972) *Planta* **108**, 333.
133. Kulaeva, O. N. and Kuznetsov, V. V. (1976) *Fiziol. Rast.* **23**, 1255.
134. Rijven, A. H. G. C. and Prakash, V. (1971) *Plant Physiol.* **47**, 59.
135. Kende, H., Dilworth, H. and Dezacks, R. (1974) in *Plant Growth Substances* (Tamaya, S., ed.). Hirokawa Publ., Tokyo.
136. Asthana, J. S. and Srivastava, H. S. (1978) *Indian J. Plant Physiol.* **21**, 150.
137. Nelson, N. and Ilan, I. (1969) *Plant Cell Physiol.* **56**, 692.
138. Sawhney, S. K. and Naik, M. S. (1973) *Indian J. Biochem. Biophys.* **10**, 199.
139. Eaglesham, A. R. J. and Hewitt, E. J. (1975) *Plant Cell Physiol.* **16**, 1137.
140. Eaglesham, A. R. J. and Hewitt, E. J. (1971) *FEBS Letters* **16**, 315.
141. Maldonado, J. M., Notton, B. A. and Hewitt, E. J. (1978) *FEBS Letters* **93**, 169.
142. Maldonado, J. M., Notton, B. A. and Hewitt, E. J. (1978) *Plant Sci. Letters* **13**, 143.
143. Losada, M. (1974) in *Metabolic Interconversion of Enzymes* (Eischler, E. H., et al., eds.). Springer, Berlin.
144. Losada, M. (1976) *J. Mol. Catal.* **1**, 245.
145. Decleire, M., Decat, W. and Bastin, R. (1976) *Z. Pflanzenphysiol.* **77**, 315.
146. Churchill, K. and Klepper, L. (1978) *Plant Physiol.* **s-294**.
147. Hofstra, J. J. (1977) *Physiol. Plant.* **41**, 65.
148. Tweedy, J. A. and Ries, S. K. (1967) *Plant Physiol.* **42**, 280.
149. Mohandas, S., Wallace, W. and Nicholas, D. J. D. (1978) *Phytochemistry* **17**, 1021.
150. Meeks, J. R. and Evans, T. R. (1978) *Plant Physiol.* **s-111**.
151. Harper, J. E. and Hageman, R. H. (1972) *Plant Physiol.* **61**, 900.
152. Galitz, D. S. (1973) *Weed Sci.* **22**, 23.
153. Srivastava, H. S. (1975) *Plant Cell Physiol.* **16**, 995.
154. Srivastava, H. S., Oaks, A. and Bakyta, I. L. (1976) *Can. J. Botany* **54**, 923.
155. Schlesier, G. (1977) *Biochim. Physiol. Pflanzen.* **171**, 511.
156. Nair, T. V. R. and Abrol, Y. P. (1978) *Indian J. Plant Physiol.* **21**, 41.
157. Sinha, S. K., Khanna-Chopra, R., Chatterji, S. R. and Abrol, Y. P. (1978) *Physiol. Plant.* **42**, 45.
158. Menary, R. C. and Jones, R. H. (1972) *Aust. J. Biol. Sci.* **25**, 531.
159. Kohl, J. G., Abel, M. and Dudel, G. (1974) *Biochim. Physiol. Pflanz.* **167**, 501.
160. Bautista, A. M., Quisumbing, E. C. and Palis, R. K. (1976) *Phillip. Agric.* **60**, 237.
161. Simmins, S. R. and Moss, D. N. (1978) *Crop Sci.* **18**, 584.
162. Murty, G. S. R. and Sirohi, G. S. (1975) *Indian J. Plant Physiol.* **18**, 60.
163. Srivastava, H. S. (1973) *Indian J. Plant Physiol.* **16**, 57.



164. Travis, R. L. and Key, J. L. (1971) *Plant Physiol.* **48**, 617.
165. Wallace, W. and Pate, J. S. (1965) *Ann. Botany* **29**, 655.
166. Hageman, R. H., Flesher, D. and Gitter, A. (1961) *Crop Sci.* **1**, 201.
167. Nicholas, J. C., Harper, J. E. and Hageman, R. H. (1976) *Plant Physiol.* **58**, 731.
168. Tischler, C. R., Purvis, A. C. and Jordon, W. R. (1978) *Plant Physiol.* **61**, 714.
169. Duke, S. H., Friedrich, J. W., Schrader, L. E. and Koukkari, W. L. (1978) *Physiol. Plant.* **42**, 269.
170. Upcroft, J. A. and Done, J. (1972) *Plant Physiol.* **42**, 280.
171. Upcroft, J. A. and Done, J. (1976) *Aust. J. Plant Physiol.* **3**, 421.
172. Cohen, A. S. and Cumming, B. G. (1974) *Can. J. Botany* **52**, 2351.
173. Steer, B. T. (1973) *Plant Physiol.* **53**, 530.
174. Steer, B. T. (1974) *Plant Physiol.* **54**, 758.
175. Steer, B. T. (1976) *Plant Physiol.* **57**, 928.
176. Bakshi, I. S., Abad-Farooqui, A. H. and Maheshwari, S. C. (1978) *Z. Pflanzenphysiol.* **90**, 165.
177. Benzioni, A. and Heimer, Y. M. (1977) *Plant Sci. Letters* **9**, 225.
178. Mattas, R. E. and Pauli, A. W. (1965) *Crop Sci.* **5**, 181.
179. Younis, M. A., Pauli, A. W., Michell, H. L. and Stickler, F. E. (1965) *Crop Sci.* **5**, 321.
180. Onweunne, I. C., Laude, H. M. and Huffaker, R. C. (1971) *Crop Sci.* **11**, 195.
181. Alofe, C. O., Schrader, L. E. and Smith, R. R. (1973) *Crop Sci.* **13**, 625.
182. Amos, J. A. and Scholl, R. L. (1977) *Crop Sci.* **17**, 445.
183. Kauffman, J. E., Beard, J. B. and Penner, D. (1971) *Physiol. Plant.* **25**, 378.
184. Magalhass, A. C., Peters, D. B. and Hageman, R. H. (1976) *Plant Physiol.* **58**, 12.
185. Travis, R. L., Huffaker, R. C. and Key, J. L. (1970) *Plant Physiol.* **46**, 800.
186. Travis, R. L., Jordon, W. R. and Huffaker, R. C. (1970) *Physiol. Plant.* **23**, 678.
187. Jordon, W. R. and Huffaker, R. C. (1972) *Plant Physiol.* **26**, 296.
188. Bilali, I. M. and Rains, D. W. (1973) *Physiol. Plant.* **28**, 237.
189. Frith, G. J. T. (1974) *Plant Cell Physiol.* **15**, 153.
190. Bornman, C. H. (1965) *S. Afric. J. Agric. Sci.* **8**, 909.
191. Johnson, C. B. (1976) *Planta* **128**, 127.
192. Shibata, M., Kobayashi, M. and Takahashi, E. (1969) *Plant Cell Physiol.* **10**, 337.
193. Nicholas, J. C., Harper, J. E. and Hageman, R. H. (1976) *Plant Physiol.* **58**, 736.
194. Fellipe, G. M., Dale, J. E. and Marriott, C. (1975) *Ann. Botany* **39**, 43.
195. Travis, R. L., Key, J. L. and Ross, C. W. (1974) *Plant Physiol.* **53**, 28.
196. Duke, S. H. and Duke, S. O. (1978) *Plant Cell Physiol.* **19**, 481.
197. Beevers, L. and Hageman, R. H. (1972) *Photophysiology* **7**, 85.
198. Chen, T. M. and Ries, S. K. (1969) *Can. J. Botany* **47**, 341.
199. Srivastava, H. S. (1974) *Indian J. Biochem. Biophys.* **11**, 230.
200. Nasrulhaque-Boyce, A. and Jones, O. T. G. (1977) *Planta* **137**, 77.
201. Williams, G. R. and Novelli, G. D. (1968) *Biochim. Biophys. Acta* **155**, 183.
202. Poulson, R. and Beevers, L. (1970) *Plant Physiol.* **46**, 315.
203. Jolly, S. O. and Tolbert, N. E. (1978) *Plant Physiol.* **62**, 197.
204. Wallace, W. (1973) *Plant Physiol.* **52**, 197.
205. Wallace, W. (1974) *Biochim. Biophys. Acta* **341**, 265.
206. Kadam, S. S., Gandhi, A. P., Sawhney, S. K. and Naik, M. S. (1974) *Biochim. Biophys. Acta* **350**, 162.
207. Yamaya, T. and Ohira, K. (1978) *Plant Cell Physiol.* **19**, 211.
208. Yamaya, T. and Ohira, K. (1976) *Plant Cell Physiol.* **17**, 633.
209. Wright, L. A., Jr. and Murphy, T. M. (1975) *Biochim. Biophys. Acta* **407**, 338.
210. Huffaker, R. C., Radin, T., Kleinkopf, G. E. and Cox, E. L. (1970) *Crop Sci.* **10**, 471.
211. Morilla, C. A., Boyer, J. S. and Hageman, R. H. (1973) *Plant Physiol.* **51**, 817.
212. Shaner, D. L. and Boyer, J. S. (1976) *Plant Physiol.* **58**, 505.
213. Bradzik, J. M., Marsh, H. V. and Harris, J. R. (1971) *Plant Physiol.* **47**, 828.
214. Plaut, Z. (1973) *Plant Soil* **38**, 81.
215. Ackerson, R. C., Kreig, D. R., Haring, C. L. and Chang, N. (1971) *Crop Sci.* **17**, 81.
216. Anikiev, U. V. and Kuramagomedov, M. K. (1975) *Fiziol. Rast.* **22**, 354.
217. Hsiao, T. C. (1970) *Plant Physiol.* **46**, 281.
218. Klepper, L. A., Flesher, D. and Hageman, R. H. (1971) *Plant Physiol.* **48**, 580.
219. Purvis, A. C., Peters, D. B. and Hageman, R. H. (1974) *Plant Physiol.* **53**, 934.
220. Stulen, I. and Lanting, L. (1978) *Physiol. Plant.* **42**, 283.
221. Blevins, D. G., Lowe, R. H. and Staples, L. (1976) *Plant Physiol.* **57**, 457.
222. Garcia-Nova, F. and Crawford, R. M. M. (1973) *New Phytol.* **72**, 1031.
223. Lambers, H. (1976) *Physiol. Plant.* **37**, 117.
224. Mifflin, B. J. (1970) *Rev. Roum. Biochim.* **7**, 53.
225. Lee, R. B. (1978) *J. Exp. Botany* **110**, 693.
226. Ambrust, D. V. and Paulson, G. M. (1973) *Commun. Soil Sci. Plant Anal.* **4**, 1971.
227. Tingey, D. T., Fites, R. C. and Wickliff, C. (1973) *Physiol. Plant* **29**, 33.
228. Purvis, A. C. (1978) *Can. J. Botany* **56**, 1540.
229. Muller, A. J. and Grafe, R. (1978) *Mol. Gen. Genet.* **161**, 67.
230. Glimelius, K., Eriksson, T., Grafe, R. and Muller, A. J. (1978) *Physiol. Plant.* **44**, 273.