

continuously (leading strand) in one direction and discontinuously (lagging strand) in the other direction. Usually, the replication termination point from the origin of replication is present in a symmetrical manner to complete the replication simultaneously from the opposite fork. The localized asymmetry referred to above, therefore, would not affect the overall symmetry between the DNA strands. DNA replication rate is an important phenomenon in the cell. During replication, though the leading and the lagging strands are being synthesized by the same replisome complex, the directional advantage of the leading strand synthesis might lead to a net delay in the synthesis of the lagging strand. To overcome this delay and complete both strands simultaneously, the cell might have adopted a strategy of maintaining the leading strand rich in keto bases and vice versa. Further work in this aspect will reveal the mechanism and reason for the leading and lagging strand compositional asymmetry within a replisome. It is pertinent to note that the composition of oligonucleotides in the genome has been analysed earlier by several workers<sup>16,17</sup>, but their objective was to analyse differential oligonucleotide compositional pattern in the genome and also how this can be used to find out alien DNA sequences in the genome. More studies on this aspect will reveal the exact reason for the symmetrical nature of the genome.

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## Evaluation of heat acclimation and salicylic acid treatments as potent inducers of thermotolerance in *Cicer arietinum* L.

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**Efficacy of heat acclimation and salicylic acid (SA) treatments in induction of thermotolerance was tested in six different genotypes of *Cicer arietinum* L. Remarkable reduction in relative injury of membranes was observed in plants pre-treated with SA in comparison to heat-acclimatized and untreated control seedlings subjected to lethal temperature treatment. Both treatments resulted in increase in protein and proline content over control seedlings, which was more significant in SA pre-treatments, with the maximum increase being recorded in ICC 4918 and 1852. Both treatments led to the induction of peroxidase (POX), ascorbate peroxidase (APOX) and catalase (CAT) activities. Activities of POX and APOX increased remarkably, while CAT showed a reduction in activity.**

**Keywords:** Thermotolerance, salicylic acid, heat acclimation, antioxidative enzymes, *Cicer arietinum*.

HIGH surface temperatures are common to soils during periods of drought. Seedlings frequently experience high temperature during emergence and establishment in many regions of the world, which leads to reduction in yield<sup>1</sup>. When

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plants are exposed to excess heat, a characteristic set of cellular and metabolic response is triggered. The heat shock is characterized by a transient expression of heat shock proteins (HSPs)<sup>2</sup>. The expressions of HSPs positively correlate with the acquisition of thermotolerance, and the over-expression of HSPs often results in enhanced thermotolerance<sup>3,4</sup>. It is well established that plants can respond defensively to heat-stress. A preliminary treatment with a moderately elevated, non-lethal temperature can temporarily render plants more resistant to a subsequent potentially lethal heat shock – this phenomenon is known as heat acclimation.

Tolerance and heat acclimation to heat-stress are of significant importance to crops. High-temperature stress invariably causes denaturation of proteins, resulting in the formation of insoluble aggregates, and hampering cell recovery after heat shock<sup>5</sup>. Hong *et al.*<sup>6</sup> reported that the ability of organisms to acquire thermotolerance to normally lethal temperature is an ancient and conserved adaptive response. Acquisition of thermotolerance is likely to be of particular importance to plants that experience daily temperature fluctuations and are unable to escape to more favourable environments.

Thermotolerance can be categorized as either inherent or acquired. Inherent thermotolerance relates to the ability of an organism to withstand, up to a certain degree, a rapid change in temperature away from the optimum. Acquired thermotolerance means the level of protection beyond the inherent thermotolerance that results from prior exposure to elevated, non-lethal temperatures or tolerance induced by many other putative signalling components like salicylic acid (SA), abscisic acid (ABA), CaCl<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, ethylene, etc.

Increased formation of reactive oxygen species is a general feature of abiotic stresses, such as extreme temperature, high light and drought<sup>7,8</sup>. In order to limit oxidative damage under stress conditions, plants have developed a series of detoxification systems which include enzymes like peroxidase, ascorbate peroxidase, catalase, superoxide dismutase, etc.<sup>9,10</sup>.

In this study, an attempt was made to induce thermotolerance in 15-day-old *Cicer* seedlings of six different genotypes, ICC 4918, ICC 4969, ICC 7344, ICC 1852, ICC 10035 and ICC 6119, raised from the seeds obtained from the Chickpea Germplasm Collection of ICRISAT, Patancheru, Andhra Pradesh. The seedlings were grown in 10" size clay pots containing steam-sterilized soils. Induction of thermotolerance was attempted by heat acclimation (42°C pre-treatment for 2–4 h) and SA foliar spray treatment. Temperature treatment was given either by direct exposure to 46°C (lethal temperature treatment for 2 h) or pre-incubation at 42°C for 2–4 h, followed by challenge with a lethal temperature. The effect of these treatments on the antioxidant enzymes and certain other biochemical components associated with temperature stress, were also studied.

SA foliar spray (100 µM/l SA solution) measuring 50 ml was sprayed on *Cicer* seedlings twice a day for three consecutive days and finally just prior to exposure to heat-stress.

The same volume of distilled water was sprayed on control plants for the same number of times. The SA pre-treated plants were then subjected to heat shock treatment along with untreated control and heat-acclimatized seedlings. The genotypic variation of thermotolerance in different genotypes was then analysed biochemically by evaluation of cell membrane stability, protein profile, proline accumulation and activities of antioxidative enzymes – peroxidase (EC.1.11.1.7), ascorbate peroxidase (EC. 1.11.1.11) and catalase (1.11.1.6).

Soluble protein was extracted from the plant tissue following the method of Chakraborty *et al.*<sup>11</sup>. Plant tissues were homogenized using 0.05 M sodium phosphate buffer (pH 7.2) containing 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, 0.5 mM MgCl<sub>2</sub> and 2 mM PMSF added during crushing and centrifuged at 4°C for 20 min at 10,000 rpm. The supernatant was used as crude protein extract and protein content was estimated following the method of Lowry *et al.*<sup>12</sup>.

SDS-PAGE analysis of total soluble protein was performed on 10% gel following the method of Sambrook *et al.*<sup>13</sup>.

Proline was extracted from the plant tissue in 3% sulphosalicylic acid and estimated following the method of Bates *et al.*<sup>14</sup>. The estimation of membrane dysfunction under stress by measuring cellular leakage from affected tissue into a medium, is used as a means of determining cell membrane stability (CMS). CMS is first calculated as a percentage and the relative injury (RI) per cent is given<sup>15</sup> by % RI = 100 – %CMS. This test was carried out with pinnules obtained from 15-day-old *Cicer* seedlings.

For extraction of enzymes, plant tissues were weighed and ground with a pestle in an ice-cold mortar with 0.05 M Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 6.9) containing 2 mM *b*-mercaptoethanol. The homogenate was centrifuged at 4°C for 20 min at 15,000 g. The supernatant was used for the activity assay of peroxidase, ascorbate peroxidase and catalase.

Peroxidase activity was assayed spectrophotometrically as increase in absorbance at 460 nm by monitoring the oxidation of O-dianisidine in the presence of H<sub>2</sub>O<sub>2</sub> by the enzyme<sup>16</sup>. Enzyme activity was expressed as change/increase in absorbance ( $\Delta A_{460}$ ) mg protein<sup>-1</sup> min<sup>-1</sup>.

Ascorbate peroxidase<sup>17</sup> activity was assayed as decrease in absorbance by monitoring the oxidation of ascorbate at 290 nm. Enzyme activity was finally expressed as change (decrease) in absorbance ( $\Delta A_{290}$ ) mg protein<sup>-1</sup> min<sup>-1</sup>.

Catalase activity was measured according to Chance and Machly<sup>18</sup> by monitoring the breakdown of H<sub>2</sub>O<sub>2</sub> at 240 nm in a UV-VIS spectrophotometer (SICO). Enzyme activity was expressed as enzyme units mg protein<sup>-1</sup>, where one enzyme unit was defined as a change of 0.01 absorbance per minute caused by the enzyme aliquot.

In the present investigation, the role of heat acclimation and SA treatments in induction of thermotolerance was investigated. The results categorized the cultivars into three main groups, namely heat-tolerant (ICC 4918 and ICC1852), moderately tolerant (ICC 4969 and ICC 6119),

**Table 1.** Effect of heat acclimation and SA pre-treatment on cell membrane stability of *Cicer* seedlings exposed to lethal temperature

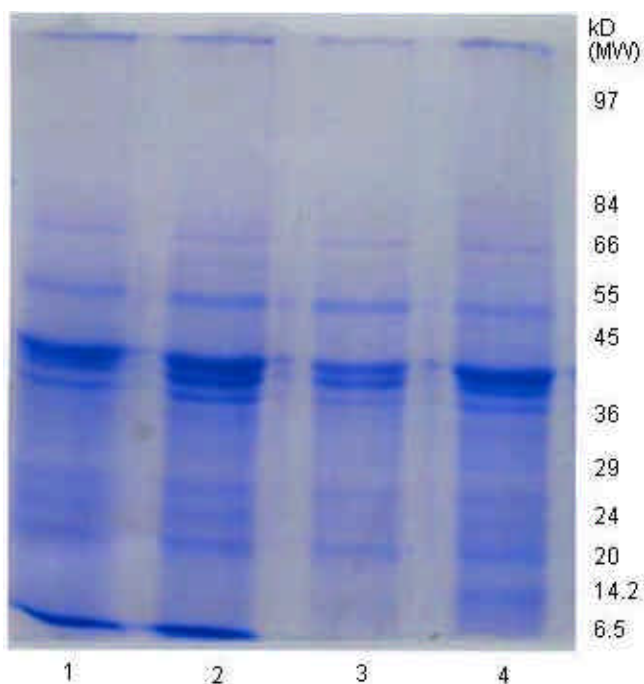
Genotype	Relative injury of membrane (in %)			
	Control	Heat acclimation	SA	Lethal
ICC 4918	27.83 ± 0.50	25.32 ± 0.49	22.63 ± 0.46	42.59 ± 0.52
ICC 4969	42.20 ± 0.54	36.18 ± 0.48	35.23 ± 0.26	63.89 ± 0.30
ICC 7344	77.86 ± 0.40	70.32 ± 0.51	60.38 ± 0.53	86.32 ± 0.19
ICC 1852	35.03 ± 0.11	33.02 ± 0.47	29.93 ± 0.44	49.65 ± 0.56
ICC 10035	69.64 ± 0.28	65.63 ± 0.23	59.84 ± 0.64	82.04 ± 0.42
ICC 6119	33.60 ± 0.67	30.03 ± 0.74	27.69 ± 0.47	55.79 ± 1.28

Values represent mean ± SE (n = 3). Values are mean of three replicates.

**Table 2.** Protein content of *Cicer* seedlings subjected to heat acclimation and SA pre-treatment and exposed to lethal temperature

Genotype	Protein content (mg/g tissue)			
	Control	Heat acclimation	SA	Lethal
ICC 4918	41.56 ± 1.82	48.46 ± 0.42	60.18 ± 3.14	38.36 ± 0.78
ICC 4969	44.84 ± 0.08	52.21 ± 0.13	60.85 ± 0.14	39.16 ± 1.50
ICC 7344	48.08 ± 1.15	50.32 ± 1.09	56.35 ± 1.34	39.63 ± 1.89
ICC 1852	46.45 ± 2.09	54.39 ± 0.19	61.17 ± 0.51	42.12 ± 0.82
ICC 10035	47.11 ± 0.44	50.29 ± 0.60	52.98 ± 0.99	34.89 ± 0.49
ICC 6119	39.70 ± 0.48	48.16 ± 0.06	53.97 ± 1.14	33.85 ± 0.98

Values represent mean ± SE (n = 3). Values are mean of three replicates.



**Figure 1.** SDS-PAGE analysis of proteins from *Cicer* seedlings subjected to heat acclimation and SA pre-treatments. Lane 1, Control; lane 2, Heat acclimation pre-treatment + lethal temperature treatment; lane 3, Lethal treatment and lane 4, SA pre-treatment + lethal temperature treatment.

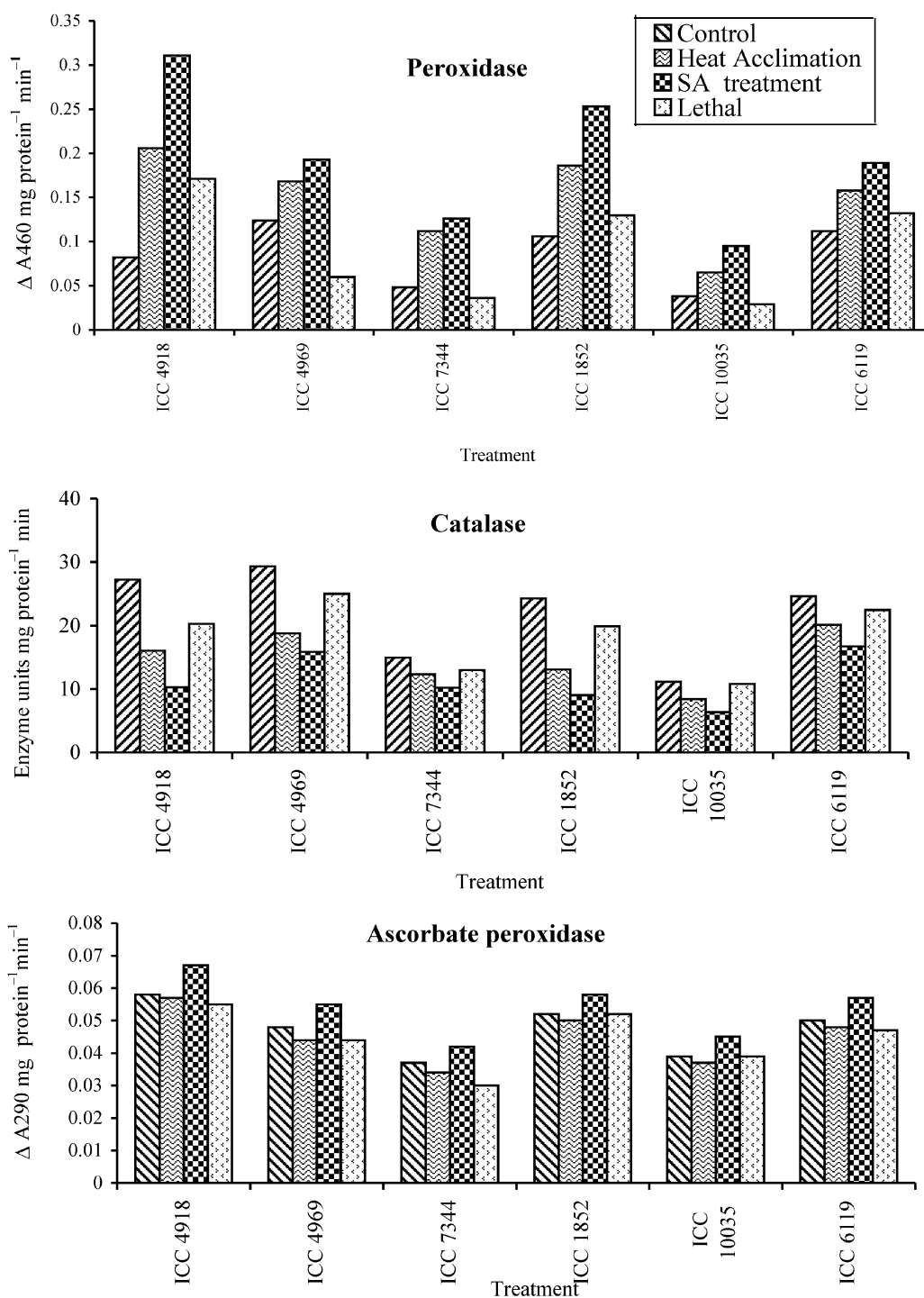
of heat acclimation treatments (2–4 h) are successful in induction of thermotolerance and SA pre-treatments confer better heat tolerance than heat-acclimation treatments.

RI of membranes was significantly high with heat-stressed plants compared to control (Table 1). Lower injury was recorded with plants pre-treated with SA than heat-acclimatized plants. Lowest injury was recorded in genotype ICC 4918, closely followed by ICC 1852 and maximum in ICC 7344. Premchandra *et al.*<sup>19</sup> and Reddy *et al.*<sup>20</sup> have reported that cell membrane stability is an indicator of drought tolerance, which is often associated with heat-stress. Higher percentage of injury under water stress has also been reported in susceptible cultivars in wheat<sup>21</sup>.

Protein content varied with type of cultivar and interestingly, maximum protein contents were recorded in ICC 7344 and 10035. Protein degradation following prolonged heat treatments was higher in ICC 6119 and ICC 4969, lower in ICC 4918 and ICC 1852 and highest in ICC 7344 and ICC 10035 (Table 2). The extent of decrease in total protein content was significantly lower in heat-tolerant cultivars than in susceptible cultivars. SDS-PAGE analysis of pre-treated seedlings subjected to lethal temperature treatments revealed the appearance of some newly synthesized proteins. A low-molecular weight protein of 14 kDa (approx) and another protein of molecular weight 36 kDa (approx) were observed in SA pre-treated plants challenged with lethal temperature (Figure 1).

Proline content increased remarkably in both treatments following lethal temperature treatment (Table 3). However,

and sensitive (ICC 10035 and ICC 7344). The results of the present finding also suggest that only long-term duration



**Figure 2.** Effect of heat acclimation and SA pre-treatment on peroxidase, catalase and ascorbate peroxidase activities in *Cicer* seedlings following heat-stress.

proline content of SA pre-treated seedlings was significantly higher than heat-acclimatized seedlings. This was more significant in the two tolerant cultivars, which also had higher constitutive proline. Increased proline content under stress has been reported earlier<sup>22</sup> and the results of the present finding further confirm these reports.

Peroxidase enzymes are known to decompose  $H_2O_2$  by oxidation of phenolic compounds and prevent lipid peroxidation of membranes. POX activity increased significantly in all genotypes tested in both treatments over control, following heat-stress. However, significant increase was recorded in relatively tolerant cultivars (ICC 4918 and 1852)

**Table 3.** Effect of heat acclimation and SA pre-treatment on proline content of *Cicer* seedlings exposed to lethal temperature

Genotype	Proline content (mg/g tissue)			
	Control	Heat acclimation	SA	Lethal
ICC 4918	0.43 ± 0.005	0.51 ± 0.005	0.56 ± 0.009	0.49 ± 0.003
ICC 4969	0.36 ± 0.003	0.42 ± 0.003	0.47 ± 0.005	0.39 ± 0.008
ICC 7344	0.29 ± 0.005	0.35 ± 0.000	0.38 ± 0.003	0.33 ± 0.008
ICC 1852	0.48 ± 0.005	0.56 ± 0.008	0.62 ± 0.005	0.53 ± 0.005
ICC 10035	0.27 ± 0.007	0.32 ± 0.005	0.35 ± 0.010	0.30 ± 0.010
ICC 6119	0.38 ± 0.003	0.43 ± 0.003	0.45 ± 0.003	0.41 ± 0.003

Values represent mean ± SE ( $n = 3$ ). Values are mean of three replicates.

than in susceptible ones (ICC 7344 and ICC 10035; Figure 2). Other investigators have also reported increase in POX activity in grasses<sup>23</sup> and in cucumber seedlings under UV-B stress<sup>24</sup>.

Heat-induced oxidative damage was reduced in plants pre-treated with SA and heat-acclimatized plants over control. This was manifested by the increased activities of POX and APOX. Activity of APOX increased over the first few hours of heat treatment, but decreased gradually on prolonged heat treatment. APOX activity registered a marginal decrease in heat-acclimatized seedlings over control. SA pre-treated seedlings, however, showed increased activity following lethal temperature treatment (Figure 2). Peltzer *et al.*<sup>25</sup> have reported that regardless of the preceding environmental conditions, APOX activities in *Fagus sylvatica* and *Coleus blumei* decreased at any assay temperature above 25°C. Their result indicates that APOX activities from stressed leaves have an unusually low-temperature optimum, regardless of whether species from the tropical or temperate zone were studied.

On the other hand, CAT activity revealed a decreasing trend in pre-treated seedlings (Figure 2). CAT scavenges H<sub>2</sub>O<sub>2</sub> by breaking it down directly to form water and oxygen, and an increase in its activity is related with increase in stress tolerance<sup>26</sup>. Previous studies have shown that response of CAT to water stress may be varied. Fu and Huang<sup>23</sup> have reported that CAT activity was not affected by mild drought in cool season grasses, but it decreased<sup>27</sup> in wheat genotype C306. The present result suggested that the decrease in CAT activity was more significant in tolerant cultivars. The increased levels of H<sub>2</sub>O<sub>2</sub> presumably stimulate CAT activity.

In conclusion, SA pre-treated and heat-acclimatized *Cicer* seedlings are better able to adapt themselves to heat-stress and offer resistance to heat than control plants. The induced heat-stress tolerance response may be directly linked to the coordinated response of antioxidative enzymes like POX, APOX, CAT, etc. The scavenging of reactive oxygen species like H<sub>2</sub>O<sub>2</sub>, superoxide ions, etc. by these enzymes plays a key role in imparting heat tolerance. The high POX activity, high proline accumulation and low RI of membranes could be directly linked with enhanced tolerance to heat

induced oxidative damage. This could be used as a biochemical marker for screening thermotolerant cultivars.

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## Nitrogen resorption in leaves of tree and shrub seedlings in response to increasing soil fertility

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**In the present study an attempt was made to examine the relationship between availability of nitrogen in soil and the resorption of nitrogen before leaf fall. For this, common tree (*Quercus leucotrichophora*, *Pinus roxburghii*, *Cupressus torulosa*, *Alnus nepalensis* and *Populus ciliata*) and shrub species (*Desmodium elegans* and *Crataegus crenulata*) of Central Himalayan forests varying in leaf lifespan and other characters were selected. Seedlings of these species were raised from current year seed crop and grown at various levels of nitrogen availability. The species differed with regard to nitrogen level up to which their biomass increased with increasing nitrogen availability. In each species, the proportional resorption of nitrogen decreased continuously with increasing nutrient level. The nutrient use efficiency also decreased with increasing nutrient level in each species. These results suggest that as the availability of a limiting nutrient increases, the mechanisms used by plants to conserve that nutrient may become less efficient.**

**Keywords:** Nitrogen resorption, soil fertility, nutrients, nitrogen availability.

THE resorption of nutrients prior to leaf fall is one of the key processes by which plants conserve them. This process reduces the likelihood of nutrient loss in litter dropped on the forest floor<sup>1,2</sup> and subsequently, the withdrawn nutrients are redeployed in new tissues, such as leaves and reproductive structures or stored for later use<sup>3</sup>. The resorption accompanied by a reduction in nutrient restitution (through leaf litter) and requirements, affords the ecosystem a certain independence from the soil and the possibility of good management of the available elements<sup>4</sup>. Furthermore, nutrient resorption during leaf senescence greatly affects litter quality, litter decomposition and nutrient release. On average, plants withdraw about 50% of leaf N and P, but the proportion withdrawn<sup>5</sup> varies widely across species, 5–80% of leaf N, and 0–95% of leaf P. Some studies suggest that with increasing availability of a limiting nutrient the mechanisms used by plants to conserve that nutrient, become less efficient<sup>6,7</sup>. However, other studies have found that increased nutrients have little effect on the efficiency of internal nutrient-conservation strategies in plants<sup>8,9</sup>. In infertile habitats, N and P in senesced leaves are reduced to lower levels than in fertile habitats<sup>3</sup>. Does it apply also to within-species trends in nutrient resorption?

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