

LIDOCAINE MODULATES GROWTH PERFORMANCE OF SOME DICOT AND MONOCOT SEEDLINGS

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SUMMARY

Local anaesthetic lidocaine caused significant inhibition of seedling growth of both monocot and dicot species investigated namely *Lens culinaris*, *Vigna mungo*, *Brassica juncea*, *Triticum aestivum*, *Hordeum vulgare* and *Sorghum bicolor*. Concomitant with reduced growth, anaesthetic treatment also lowered the extent of seed reserve mobilization to the seedling, indicated by higher levels of left over seed dry weights analysed in two representative species. A species-specific lidocaine-induced anthocyanin production was observed in case of *V. mungo* cotyledons. All anaesthetic effects intensified with its concentration, although not always strictly in proportion to the dosage increment. These modulations being long-term and irreversible would obviously involve an altered gene expression induced by a local anaesthetic agent.

Key words: Lidocaine, seedling growth, seed reserve mobilization.

INTRODUCTION

Local anaesthetics (LAs) are drugs that block conduction of nerve impulse resulting in both sensory and motor paralysis. Except cocaine that occurs naturally, all other clinically used anaesthetics are synthesized ones (Ritchie and Greene 1991). LAs bring about their effect via blockade of voltage-dependent sodium channels by binding to intra-pore receptors on the membranes (Wagner *et al.* 1991, Li *et al.* 1999).

Very few attempts have been made so far to study the influence of LAs on plant systems. Such unrelated studies were all done with lidocaine (2-diethylamino-N-[2, 6-dimethylphenyl]-acetamide), an amide type of highly potent anaesthetic agent (Courtney and Strichartz 1987, Ritchie and Green 1991). It has been shown to stimulate photosynthesis in *Spinacea oleracea* (Wu and Berkowitz 1991) and desensitize plants of *Mimosa pudica* to exhibit

leaflet movements in response to external stimuli (Milne and Beamish 1999). Recently lidocaine has been shown to influence even growth and differentiation responses like inhibition of epiphyllous bud differentiation in *Kalanchoe pinnata* (Sawhney and Sawhney 2002) and root differentiation in hypocotyl cuttings of *Vigna mungo* (Rani and Sawhney 2003). It was, therefore, considered of interest to study the effect of lidocaine, on early seedling growth in plant species belonging to both monocots and dicots, in order to arrive at some generalization.

MATERIALS AND METHODS

Seeds of *Lens culinaris*, *Vigna mungo*, *Brassica juncea*, *Triticum aestivum*, *Hordeum vulgare* and *Sorghum bicolor* were used for experimentation. Healthy and uniform seeds were thoroughly washed, surface sterilized with ethanol and finally rinsed with

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autoclaved sterile water before planting. Batches of twenty seeds of each species were planted separately in petridishes having 0.8% agar, either left unsupplemented to serve as control or supplemented with lidocaine hydrochloride (Sigma-Aldrich) at concentrations of 2.5, 5, 10, 15 and 20 mM. Three replicates were maintained for each treatment. The petridishes were kept under continuous illumination in a growth chamber maintained at $25 \pm 2^\circ\text{C}$.

Observations on different growth attributes, *i.e.* maximum root length, root number and shoot length were recorded after six days of seed planting. As axis growth in monocot species was negligible, length of 2nd leaf was measured instead of shoot length. For each parameter, the relative growth (values relative to control taken as 100) as well as anaesthetic-caused relative inhibition (reciprocals of relative growth values) were also calculated.

Dry weights of seeds in case of two representatives, namely *L. culinaris* (dicot) and *T. aestivum* (monocot) were recorded prior to planting and again after six days of planting for different anaesthetic concentrations. For this, three batches of 20 seeds each, were kept in oven at 60°C for 48 h and weighed. The percent seed reserves consumed (mobilized) as well as the left over seed mass were calculated. The data were subjected to statistical analysis by calculating their $\text{SE} \pm$ mean values.

RESULTS AND DISCUSSION

The emerging seedlings of *L. culinaris*, *V. mungo* and *B. juncea* produced a single primary root whereas those of *T. aestivum*, *H. vulgare* and *S. bicolor* differentiated 3-7 adventitious roots over a period of 6 days (Table 1). Treatment with lidocaine caused a significant inhibition of root differentiation, although in a manner different for the two groups. Thus, whereas anaesthetic did not affect the emergence of single primary root up to 10 mM, and completely checked it at 20 mM levels in dicot species, it brought about a gradual reduction in root number with its rising concentration up to 15 mM and very significantly at 20 mM level, in monocots.

The maximum mean root length of 3.85 to 5.50 cm produced by dicot and 5.24 to 7.10 cm, by monocot

seedlings were also reduced significantly in all cases with lidocaine treatment and the effect increased with concentration (Table 1).

The 6 days old dicot seedlings attained a cumulative mean shoot length of 1.50 to 3.40 cm. Treatment with lidocaine caused a significant inhibition of extension growth in all cases, the effect increasing with concentration used but without exhibiting any regular dose-dependent effect. A complete inhibition of seedling growth was observed at 15 and 20 mM dose levels in all cases, except for *V. mungo* where only the latter concentration could bring the effect. Similarly, in monocot seedlings, the cumulative length of 2nd leaf was significantly reduced with lidocaine treatment, the effect increasing with concentration in all cases (Table 2).

The similarity of lidocaine-invoked responsiveness of various growth attributes among species representing two basic angiospermic groups having distinct architectural strategies pointed to an identical mode of action for the anaesthetic agent. The extent and manner of lidocaine-caused inhibition of root extension growth suggests a common and thus unifying growth parameter for all species (Fig. 1). Obviously, the universal inhibition of growth responses ought to be mediated through blockade of gross cellular activities like cell division and enlargement. This is in conformity with earlier reports for similar lidocaine-caused inhibition of various attributes of epiphyllous bud differentiation in *K. pinnata* (Sawhney and Sawhney 2002) and root differentiation in *V. mungo* (Rani and Sawhney 2003).

The observation that lidocaine-treated seedlings were stunted and retained larger attached seeds, prompted to check the influence of anaesthetic on seed reserve mobilization for a representative each of monocot and dicot species. Of the initial 42.8 and 33.5 mg seed dry weights in untreated *L. culinaris* and *T. aestivum*, respectively, 53 and 60% were exhausted within 6 days. However, with lidocaine treatment the extent of seed reserves consumed got reduced significantly as indicated by the enhanced left over seed dry weights, the effect increasing with concentration. Thus, with 15 mM lidocaine, the values for seed reserve consumption were as low as 19 and 17% respectively, in *L. culinaris* and *T. aestivum* (Fig. 2). Since, cotyledons/endosperm are

Table 1. Effect of lidocaine on root differentiation in some dicot and monocot seedlings. The figures in parentheses are values relative to their respective controls taken as 100.

Plant species	Lidocaine concentration (mM)					
	0	2.5	5	10	15	20
Number of roots differentiated						
<i>L. culinaris</i>	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
<i>V. mungo</i>	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
<i>B. juncea</i>	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
<i>T. aestivum</i>	5.66±0.51 (100)	5.25±0.48 (92.76)	5.25±0.24 (92.76)	5.24±0.16 (92.58)	4.66±0.12 (82.33)	1.00±0.01 (17.67)
<i>H. vulgare</i>	6.70±0.32 (100)	6.60±0.28 (98.51)	6.56±0.32 (97.91)	6.11±0.24 (91.19)	4.70±0.26 (70.15)	1.00±0.21 (14.92)
<i>S. bicolor</i>	3.10±0.12 (100)	3.00±0.14 (96.77)	2.00±0.12 (64.52)	2.00±0.21 (64.52)	1.00±0 (32.26)	1.00±0 (32.26)
Maximum root length						
<i>L. culinaris</i>	3.85±0.18 (100)	3.01±0.21 (78.18)	2.37±0.14 (61.56)	1.02±0.32 (26.49)	0 (0)	0 (0)
<i>V. mungo</i>	4.50±0.23 (100)	3.37±0.18 (74.89)	1.51±0.12 (33.55)	1.25±0.22 (27.78)	0.60±0.13 (13.33)	0 (0)
<i>B. juncea</i>	5.50±0.42 (100)	5.00±0.43 (90.91)	3.00±0.16 (54.54)	0.30±0.21 (5.45)	0 (0)	0 (0)
<i>T. aestivum</i>	7.10±0.38 (100)	7.10±0.53 (100)	5.67±0.22 (79.86)	1.43±0.4 (20.14)	0.45±0.31 (6.34)	0 (0)
<i>H. vulgare</i>	5.24±0.31 (100)	5.24±0.21 (100)	2.25±0.21 (42.94)	0.41±0.12 (7.82)	0.10±0.16 (1.91)	0.01±0 (0.19)
<i>S. bicolor</i>	5.32±0.24 (100)	4.21±0.34 (79.14)	3.40±0.16 (63.91)	1.70±0.25 (31.95)	0.30±0.23 (5.64)	0.10±0.01 (1.88)

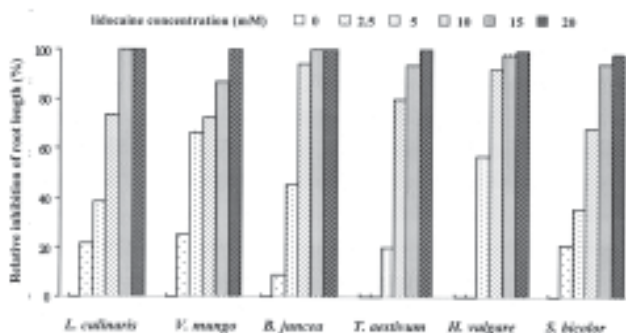
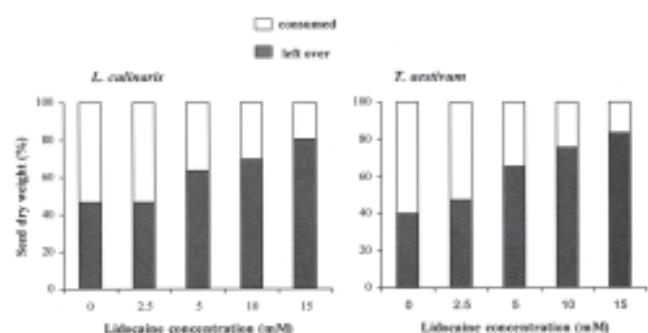
Table 2. Effect of lidocaine on shoot growth in some dicot and monocot seedlings. The figures in parentheses are values relative to their respective controls taken as 100.

Plant species	Lidocaine concentration (mM)					
	0	2.5	5	10	15	20
Length of shoot axis (dicots)						
<i>L. culinaris</i>	3.40±0.12 (100)	2.05±0.11 (60.29)	1.45±0.18 (42.65)	0.45±0.21 (13.23)	0 (0)	0 (0)
<i>V. mungo</i>	2.98±0.23 (100)	2.58±0.16 (86.58)	2.02±0.23 (67.79)	1.40±0.18 (46.98)	0.94±0.14 (31.54)	0 (0)
<i>B. juncea</i>	1.50±0.14 (100)	1.00±0.11 (66.67)	0.70±0.12 (46.67)	0.50±0.12 (33.33)	0 (0)	0 (0)
Length of 2nd leaf (monocots)						
<i>T. aestivum</i>	8.70±0.21 (100)	8.50±0.31 (97.70)	6.77±0.23 (77.82)	3.23±0.18 (37.13)	0.69±0.23 (7.93)	0 (0)
<i>H. vulgare</i>	8.21±0.32 (100)	8.24±0.26 (100.36)	7.81±0.12 (95.13)	3.12±0.14 (38.00)	2.51±0.16 (30.57)	0.01±0 (0.12)
<i>S. bicolor</i>	2.51±0.18 (100)	2.53±0.22 (100.80)	2.19±0.14 (87.25)	1.22±0.16 (48.60)	0.80±0.18 (31.87)	0.50±0.21 (19.92)

primary contributors of nutrients during early seedling establishment (Monnier 1982, Gambley and Dodd 1991, Saluja and Sawhney 2003, Shutov *et al.* 2003), these effects could be interpreted only if it is assumed that lidocaine treatment significantly reduced the extent of reserve mobilization from seeds to the growing seedling, and to more or less similar extent for the two storage organs. Whether this anaesthetic effect was brought about by an inhibition of synthesis or activity of hydrolases

or otherwise, remains to be seen. In this context, lidocaine-caused inhibition of early seedling growth attributes could well be a consequence rather than a cause of such metabolic modulations.

Although the growth performance of all the six plant species was similarly inhibited (Fig. 3), it was only in *V. mungo* that a characteristic cotyledonary behavior was observed in response to lidocaine application. In untreated

**Fig. 1.** Lidocaine – invoked relative inhibition of root length (%) in some dicot [*L. culinaris*, *V. mungo* and *B. juncea*] and monocot [*T. aestivum*, *H. vulgare* and *S. bicolor*] seedlings raised for 6 days in media supplemented with 0, 2.5, 5, 10, 15 and 20 mM anaesthetic.**Fig. 2.** Effect of lidocaine on percent seed reserves consumed vs. left over seed mass (dry weight basis) in dicot *L. culinaris* and monocot *T. aestivum*, 6 days after planting.

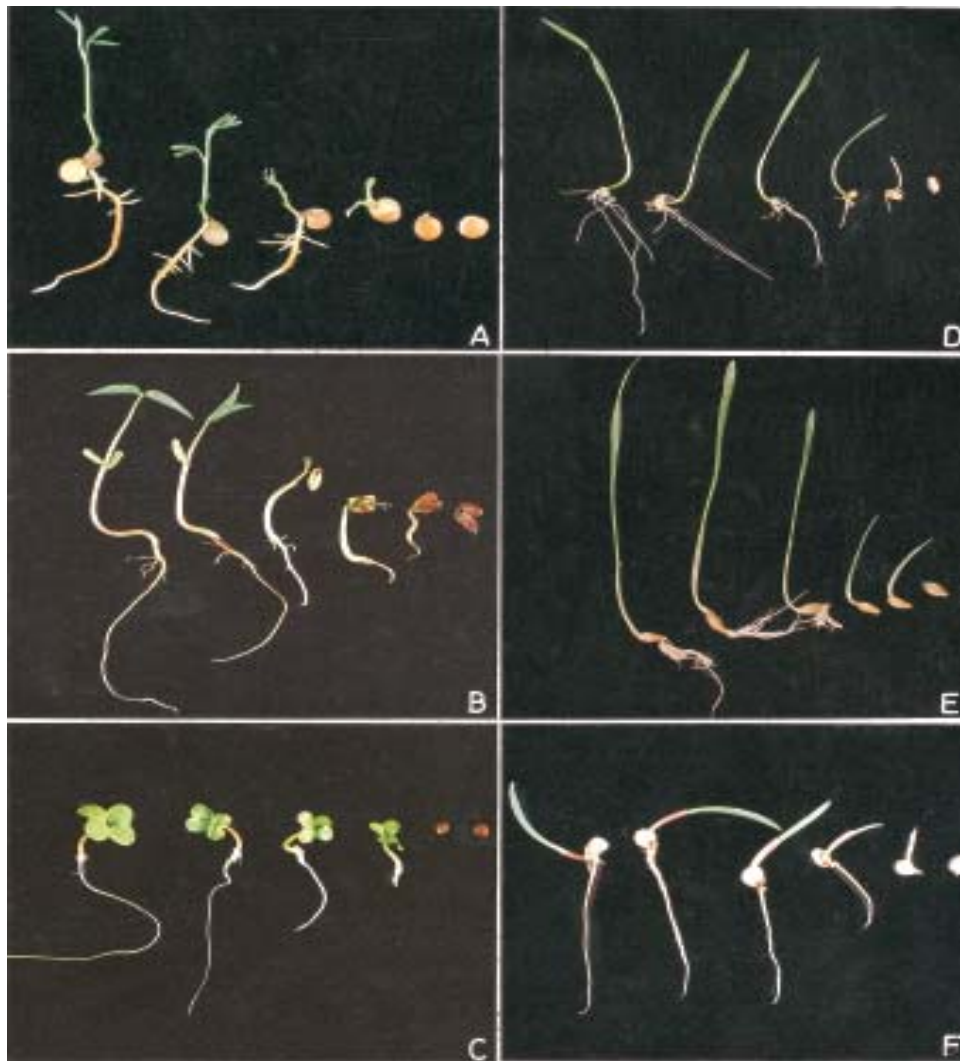


Fig. 3. Effect of lidocaine on early seedling growth in some dicots [*L. culinaris* (A), *V. mungo* (B) and *B. juncea* (C)] and monocots [*T. aestivum* (D), *H. vulgare* (E) and *S. bicolor* (F)] raised for 6 days in media supplemented with 0, 2.5, 5, 10, 15 and 20 mM anaesthetic (from left to right).



Fig. 4. Effect of lidocaine on anthocyanin distribution (visual) in retained cotyledons of *V. mungo* seedlings receiving 0, 2.5, 5, 10, 15 and 20 mM anaesthetic supplemented in the media. In each case, one cotyledon was detached after 6 days and photographed (magnified to show details). Necrosis was visible at the highest concentration only

6 days old seedling the attached cotyledons were oblong, boat shaped with incurved margins and greenish with an occasional tiny anthocyanin spot. In seedlings receiving lidocaine, the cotyledons became swollen, the margins uncurving and yellowish green with anthocyanin spreading to the central region in patches. The anthocyanin containing areas got larger with increasing anaesthetic concentration to eventually cover the entire cotyledon at 15 mM level (Fig. 4). At this dosage cotyledons also showed necrosis. Since lidocaine suppressed all facets of seedling growth expectedly by generating stressful conditions, a consequent enhanced synthesis of anthocyanin would be in accord with its reported accumulation in specific tissues experiencing diverse stresses (Mol *et al.* 1996, Trull *et al.* 1997, Chalker-Scott 1999, Mendez *et al.* 1999, Merzlyak and Chivkunova 2000). However, as this response was exhibited only by *V. mungo*, it could be taken as a species-specific potentiation of a metabolic expression towards lidocaine.

All the above effects are long-term irreversible modulations that would obviously involve an altered gene expression induced by a local anaesthetic agent. These responses are unlike the short-term and reversible blockade of nerve conduction seen in animal systems (Strichartz and Ritchie 1987) or the motor mechanism involved in leaf movements of *Mimosa pudica* (Milne and Beamish 1999, Shepherd 1999) but more in line with lidocaine-caused inhibition of various attributes of epiphyllous bud differentiation observed in *K. pinnata* (Sawhney and Sawhney 2002) and root differentiation in *V. mungo* (Rani and Sawhney 2003).

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