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EFFECTS OF THE SODIUM AND CALCIUM CONCENTRATIONS ON THE *IN VITRO* GROWTH OF *ATRIPLEX HALIMUS* L. PLANTLETS

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ABSTRACT: The objective of this work is to analyse the sodium (Na^+) and calcium (Ca^{++}) importance in the *in vitro* morphogenesis of a halophile species the *Atriplex halimus*. The experiments have been made on plantlets coming from a seed population collected in a natural area (Algeria). Twenty medium, all different by their sodium chloride (NaCl) and calcium chloride ($CaCl_2$) content, have been tested. For five Na^+ concentrations, four $[Na^+]/[Ca^{++}]$ ratios have been tested. The $[Na^+]/[Ca^{++}]$ ratio has a significant effect on the studied criteria (stem length, longest root length and leaf number). The ratio equal to two is the one which gives the lowest values and the ratio equal to one (for the same $[Na^+]$ quantity and a double quantity of $[Ca^{++}]$) gives the strongest ones. A new doubling of the $[Ca^{++}]$ (ratio equal to 0.5) induces a decrease of the values. These results are remarkable if we take into account the low cation concentrations used in the experiments. The morphogenetical events observed here will be explained when results about cation ratios inside the plant and the enzyme activity will have been obtained.

INTRODUCTION

Atriplex halimus is a very important halophytic as a fodder plant in Mediterranean arid zones. Its *in vitro* multiplication has been made to get homogeneous and resistant populations.

Benrebiha et al. (1992) observed that the macroelement composition of the synthetic medium has a decisive importance on organogenesis of seedlings even at low concentrations.

This study presents the effects of different ratios between the concentrations of Na^+ and Ca^{++} on the growth and development of young *Atriplex halimus* plants. Le Saos and Binet (1987) have shown an effect of the interaction between Na^+ and Ca^{++} on the growth of another halophytic (*Suaeda maritima*). We used here precise values of the ratio between Na^+ and Ca^{++} for different Na^+ concentrations.

MATERIAL AND METHODS

Plant Material

The *Atriplex halimus* seeds have been collected at Djelfa (Algeria) in December 1990. They have been kept in darkness in laboratory conditions.

Methods

The seeds were decorticated to take off the fruit envelopes. Seeds were sterilised during 20 minutes in a 50 % aqueous calcium hypochlorite solution with detergent (Domestos). Seeds were placed in Petri dishes for germination with wet filter paper at 25°C constant and with a fluorescent illumination of 25 $\mu E/s/m^2$. The photoperiod was 12 hours. On the fourth imbibition day, the mean value of the plantlet length was 1.5 cm. The plantlets were subcultured (one per test tube) on the different medium.

Synthetic Medium: The twenty tested media were only different by their content in NaCl and $CaCl_2$ (Table 1). In all of them there was: $NaH_2PO_4 \cdot 2H_2O$ (15 mg/L), KNO_3 (300 mg/L), $(NH_4)_2SO_4$ (13.4 mg/L), $MgSO_4 \cdot 7H_2O$ (50 mg/L), Murashige-Skoog microelements (1962), Morel vitamins (1951), saccharose (20 g/L), EDTA-Fe (0.1 mM/L) and 8 g/L of agar (Bactoagar-Difco). The final pH was set to 5.8.

Principle of the Result Analysis: Measurements were made after one month of culture. The parameters were: stem length, number of pair of leaves, and length of the longest root. Each experiment corresponds to 20 kinds of synthetic media. Three repetitions of 24 individuals each were made for each kind of medium. For all parameters the results were analysed as a split-plot design (treatment and sub-treatment) according to the following model:

TABLE II : Effect of different Na^+ and Ca^{++} Concentrations in the Synthetic Medium on the *Atriplex halimus* Plantlet Growth.

Treatment N°	$[Na^+]$ in mM	$[Ca^{++}]$ in mM	$[Na^+]/[Ca^{++}]$	Stem length in cm	Pairs of leaves	Longest root length in cm
1	0.1	0.03	3	1.83 ± 0.25	2.15 ± 0.03	1.11 ± 0.21
2	"	0.05	2	1.51 ± 0.29	2.10 ± 0.12	1.13 ± 0.14
3	"	0.10	1	2.14 ± 0.40	2.63 ± 0.49	1.21 ± 0.14
4	"	0.20	0.5	1.80 ± 0.13	2.35 ± 0.33	1.17 ± 0.22
5	0.5	0.17	3	2.38 ± 0.33	2.67 ± 0.62	1.41 ± 0.25
6	"	0.25	2	1.46 ± 0.16	2.32 ± 0.25	1.13 ± 0.15
7	"	0.50	1	2.39 ± 0.09	2.95 ± 0.54	1.50 ± 0.08
8	"	1.00	0.5	2.02 ± 0.30	2.64 ± 0.35	1.37 ± 0.11
9	0.78	0.26	3	2.00 ± 0.07	2.50 ± 0.29	1.18 ± 0.08
10	"	0.39	2	1.74 ± 0.58	2.51 ± 0.07	1.24 ± 0.10
11	"	0.78	1	2.71 ± 0.67	3.37 ± 0.86	1.51 ± 0.03
12	"	1.56	0.5	2.10 ± 0.13	2.58 ± 0.11	1.53 ± 0.23
13	0.87	0.28	3	1.82 ± 0.03	2.39 ± 0.41	1.29 ± 0.31
14	"	0.44	2	1.88 ± 0.23	2.39 ± 0.27	1.29 ± 0.14
15	"	0.87	1	2.62 ± 0.58	3.21 ± 0.97	1.48 ± 0.14
16	"	1.74	0.5	2.19 ± 0.66	2.82 ± 0.46	1.50 ± 0.48
17	1.00	0.30	3	1.86 ± 0.11	2.37 ± 0.55	1.24 ± 0.29
18	"	0.50	2	1.70 ± 0.06	2.69 ± 0.73	1.32 ± 0.04
19	"	1.00	1	2.54 ± 0.09	3.22 ± 0.48	1.64 ± 0.05
20	"	2.00	0.5	2.10 ± 0.26	2.75 ± 0.10	1.54 ± 0.37

The global analysis points out that commonly the ratio of 2 gives the lowest values and the ratio of 1 (which is relative to a doubling of the Ca^{++} quantity) gives the highest values. Another doubling of the Ca^{++} quantity (when the $[Na^+]/[Ca^{++}]$ ratio is changed from 1 to 0.5) induces a decrease of the values.

Stem Length

The analysis of the results (Table III) points out that the Na^+ concentration in the medium, without considering its interrelations with Ca^{++} concentration, does not induce a significant change in the stem length. Nevertheless, the highest increase of the length is obtained for the concentration of 0.78 mM (Figure 1). By contrast, the $[Na^+]/[Ca^{++}]$ ratio gives a significant effect at the 1% risk level. Figure 2 shows that for all tested Na^+ concentrations, there is always an increase of the stem length when the ratio $[Na^+]/[Ca^{++}]$ is changed from 2 to 1. The highest

TABLE III : Variance Analysis for the Stem Length

Origin of the variation	d.f	S.S	M.S	F
<u>Treatment</u>				
Na^+	4	0.80	0.20	1.82
Repetition	2	0.30	0.15	1.36
Residual effect	8	0.88	0.11	
<u>Sub-Treatment</u>				
$[Na^+]/[Ca^{++}]$	3	5.15	1.72	15.64 **
$[Na^+]x[Na^+]/[Ca^{++}]$	12	1.09	0.09	0.82
Residual effect	30	3.44	0.11	

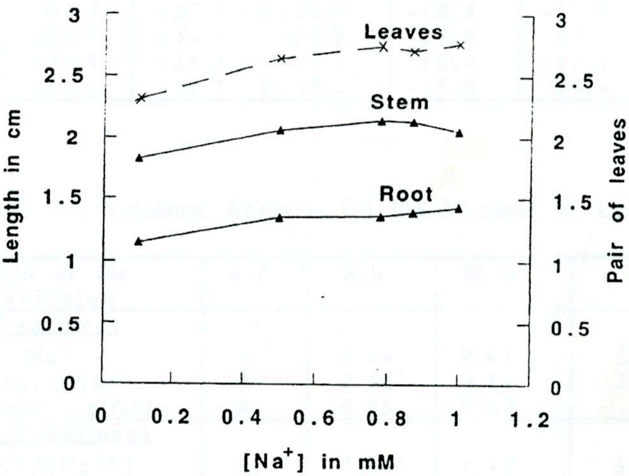

FIGURE 1 : Effect of Different Na^+ Concentrations on the *Atriplex halimus* Plantlet Growth

relative increase (+63.7%) is obtained for 0.5 mM Na^+, whereas the changing of the ratio variation from 1 to 0.5 induces a decrease of the values and the strongest decrease (-22.5%) is observed for 0.8 mM Na^+ (Table IV). For all Na^+ concentrations, the highest values of the stem length are obtained for a ratio

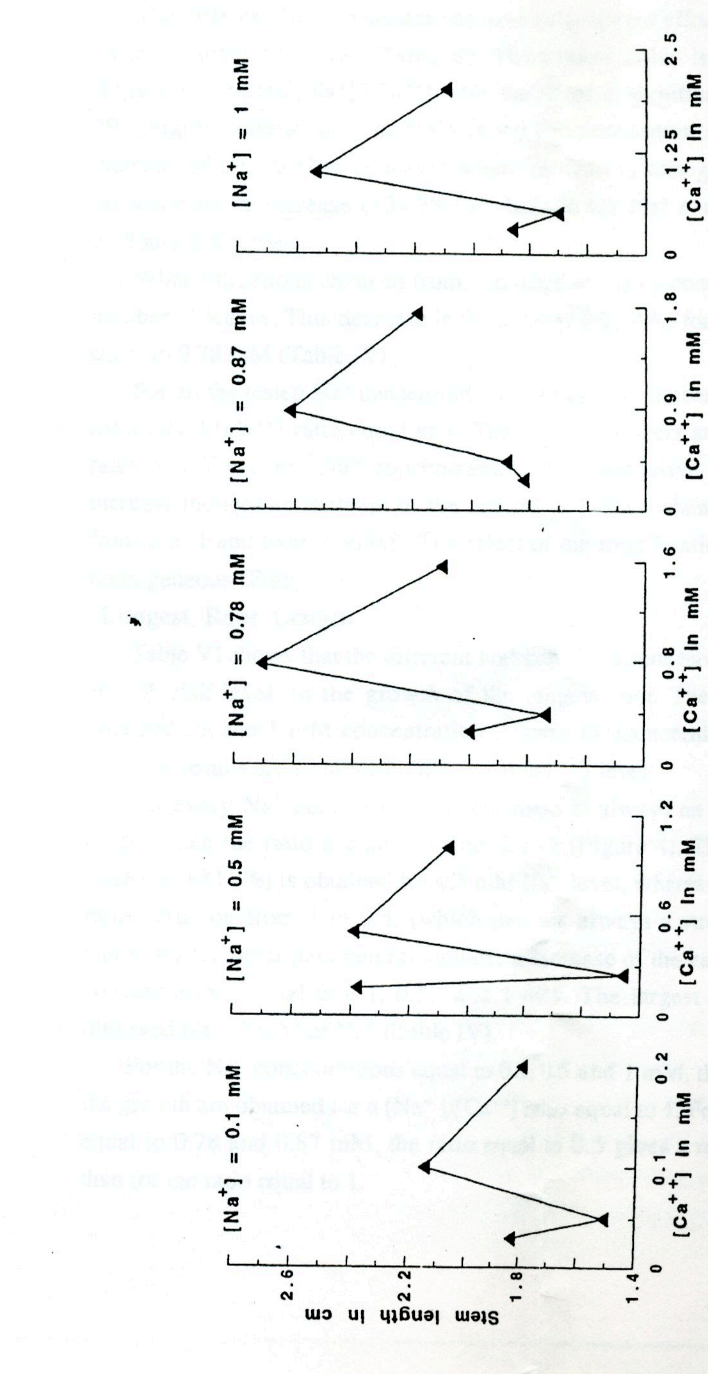

FIGURE 2 : Effect of Different Ratios [Na+]/[Ca++] on the Stem Length of *Atriplex halimus* Plantlets.

TABLE IV : Relative Variations (in %) for the Length of the Stem and the Longest Root, and for the Number of Leaves Pair, after the doubling of the Calcium Quantity and the Decrease of the Ratio $[Na^+]/[Ca^{++}]$ from 2 to 1, and from 1 to 0.5 and that for every Tested Sodium Concentrations.

$[Na^+]$ in mM	Variation % for the criteria when $[Na^+]/[Ca^{++}]$ is changing from 2 to 1			Variation % for the criteria when $[Na^+]/[Ca^{++}]$ is changing from 1 to 0.5		
	stem length	leaf number	root length	stem length	leaf number	root length
0.10	+41.7	+25.2	+7.1	-15.9	-10.6	-3.3
0.50	+63.7	+27.1	+32.7	-15.5	-10.5	-8.7
0.78	+55.7	+34.3	+21.8	-22.5	-23.4	+1.3
0.87	+39.4	+34.3	+14.7	-16.4	-12.1	+1.3
1.00	+49.4	+19.7	+24.2	-17.3	-14.6	-6.1

TABLE V : Variance Analysis for the Number of Leaf Pairs

Origin of the variation	d.f	S.S	M.S	F
<u>Treatment</u>				
Na^+	4	1.66	0.415	5.93 *
Repetition	2	0.38	0.19	2.71
Residual effect	8	0.56	0.07	
<u>Sub-Treatment</u>				
$[Na^+]/[Ca^{++}]$	3	4.43	1.48	5.48**
$[Na^+]x[Na^+]/[Ca^{++}]$	12	0.77	0.064	0.24
Residual effect	30	8.18	0.27	

$[Na^+]/[Ca^{++}]$ equal to 1. The lowest values correspond to a ratio $[Na^+]/[Ca^{++}]$ equal to 2. For every Na^+ concentration the gradual increase of Ca^{++} induces a decrease of the stem length when the $[Na^+]/[Ca^{++}]$ ratio is changed from 3 to 2. Then there is an increase of the length when the ratio is changed from 2 to 1, and there is another decrease of the parameter when the ratio is changed from 1 to 0.5. The only exception to this behaviour is when the ratio changes from 3 to 2 for 0.87 mM of Na^+.

Number of Leaves

The different Na^+ concentrations have a significant effect at the 5% risk level on the number of leaves (Table V). The highest value is obtained for 1 mM (Figure 1). For the $[Na^+]/[Ca^{++}]$ ratio, the effect is significant for a risk level of 1%. Figure 3 shows that for every tested Na^+ concentration, there is always an increase of the number of leaves when the ratio is changed from 2 to 1. The highest relative increase (+34.3%) is obtained for Na^+ concentrations equal to 0.78 and 0.87 mM.

When the ratio is changed from 1 to 0.5, there is by contrast a decrease in the number of leaves. This decrease is the greatest (-23.4%) for a Na^+ concentration equal to 0.78 mM (Table IV).

For all the tested Na^+ concentrations, the highest number of leaves is obtained for a $[Na^+]/[Ca^{++}]$ ratio equal to 1. The lowest numbers are obtained when the ratio is 2. For every Na^+ concentration, the progressive Ca^{++} concentration increase induces an increase of the number of leaves when the ratio is changed from 2 to 1 and from 1 to 0.5. The effect of the modification from 3 to 2 is not homogeneous (Figure 3).

Longest Root Length

Table VI shows that the different Na^+ concentrations have a significant effect at 5 % risk level on the growth of the longest root. The highest increase is obtained for the 1 mM concentration (Figure 1). Concerning the $[Na^+]/[Ca^{++}]$ ratio, the results are significant different at the 1% level.

For every Na^+ concentration level, there is always an increase of the root length when the ratio is changed from 2 to 1 (Figure 4). The strongest relative increase (+32.7%) is obtained for 0.5 mM Na^+ level, whereas the changing of the ratio variation from 1 to 0.5, (which induces always a strong decrease of the values for the other parameters), induces a decrease of the values just for the Na^+ concentrations equal to 0.1, 0.5, and 1 mM. The largest decrease (-8.7%) is observed for 0.5 mM of Na^+ (Table IV).

For the Na^+ concentrations equal to 0.1, 0.5 and 1 mM, the highest values for the growth are obtained for a $[Na^+]/[Ca^{++}]$ ratio equal to 1. For the concentrations equal to 0.78 and 0.87 mM, the ratio equal to 0.5 gives a result slightly higher than for the ratio equal to 1.

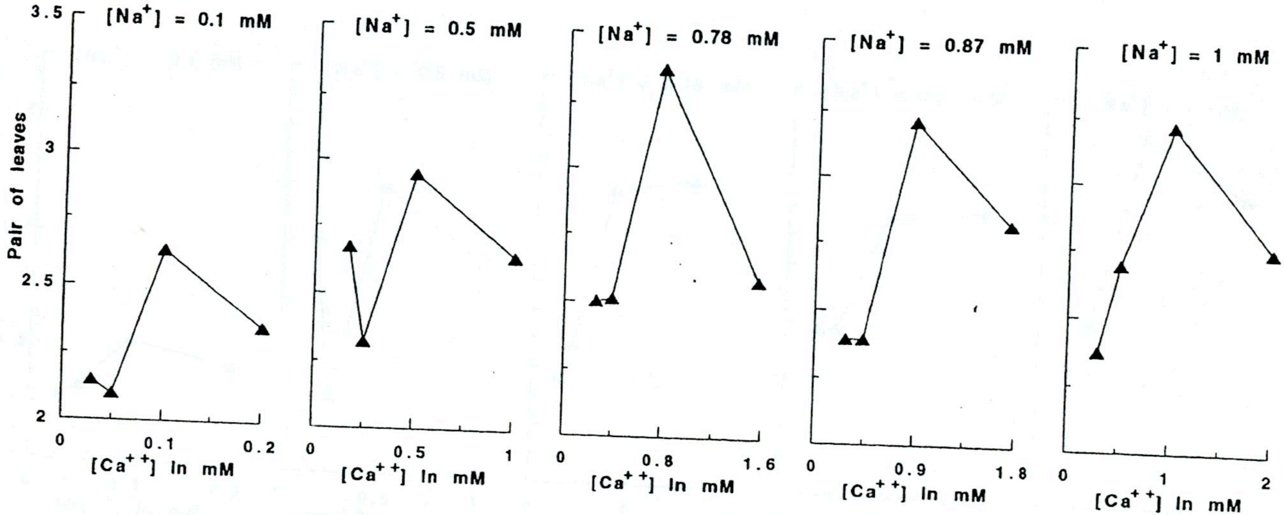

FIGURE 3 : Effect of Different Ratios [Na+]/[Ca++] on the Pair Leaves Number of *Atriplex halimus* Plantlets.

TABLE VI : Variance Analysis for the Longest Root Length

Origin of the variation	d.f	S.S	M.S	F
Treatment				
Na^+	4	0.56	0.14	4.67 *
Repetition	2	0.03	0.015	0.50
Residual effect	8	0.23	0.03	
Sub-Treatment				
$[Na^+]/[Ca^{++}]$	3	0.69	0.23	4.60**
$[Na^+]x[Na^+]/[Ca^{++}]$	12	0.29	0.024	0.48
Residual effect	30	1.54	0.05	

The highest mean value (1.64 cm) is obtained for a Na^+ and Ca^{++} concentrations equal to 1 mM, and the lowest value is obtained for a Na^+ and Ca^{++} concentrations equal to 0.1 mM and 0.03 mM, respectively.

DISCUSSION AND CONCLUSION

Our results show that among the principal events, there is a stimulation of the biological parameters when the Ca^{++} concentration is doubled and the Na^+ concentration is unchanged, (the $[Na^+]/[Ca^{++}]$ ratio is changed from 2 to 1). This is true for every tested Na^+ concentration.

Another very significant phenomenon is the decrease of the same parameters when another doubling of the Ca^{++} concentration occurs for all the five tested Na^+ concentrations (the $[Na^+]/[Ca^{++}]$ ratio is changed from 1 to 0.5).

Our results show that the variation of the absolute concentrations of Na^+ and Ca^{++} have a little effect on the measured parameters compared to the variation of the $[Na^+]/[Ca^{++}]$ ratio.

In order to explain the reproductibility of this phenomenon at five different Na^+ concentrations, and the statistical significance of the analysis, we must take into account the fact that the differences in the morphogenetic events are induced by little variations in the Na^+ and Ca^{++} concentrations (around 0.1 mM). Concentrations used in mineral nutrition studies of halophytic plants are usually much higher (Le Saos and Binet, 1987; Osmond, 1966; Mozafar et al., 1970) .

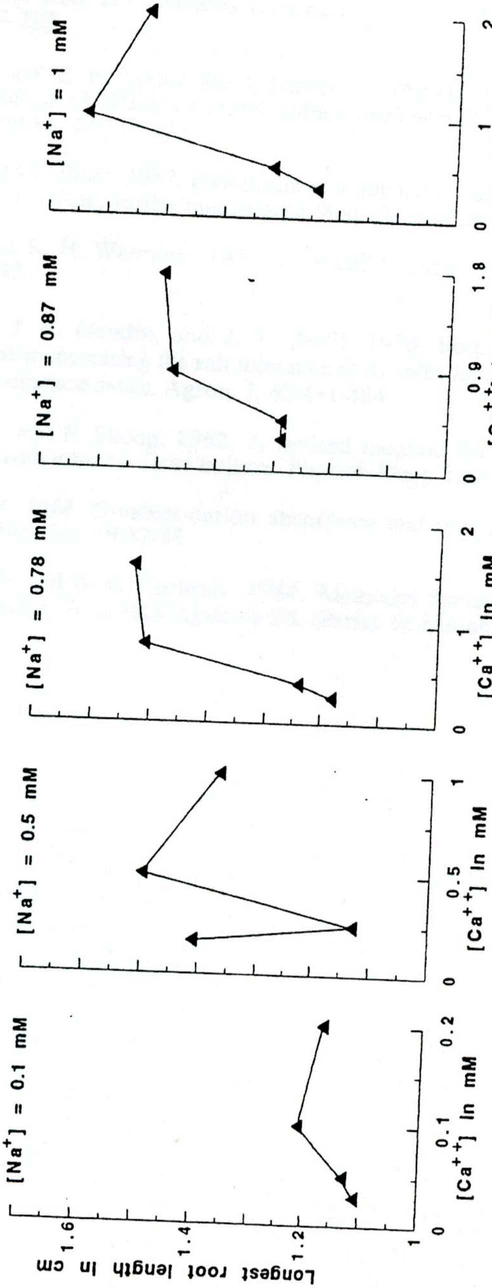

FIGURE 4 : Effect of Different Ratios [Na+]/[Ca++] on the Longest Root Length of *Atriplex halimus* Plantlets.

The effects are particularly obvious, as for instance, the stem length shows an increase of 85% between the smallest mean value (1.46 cm) and the highest mean value (2.71 cm), obtained for an increase of Na^+ and Ca^{++} concentrations of 0.28 mM and 0.53 mM, respectively (Figure 2), with all the other parameters remaining constant. The smallest value is relative to the $[Na^+]/[Ca^{++}]$ ratio equal to 2 and the strongest for the ratio equal to 1.

Mozafar et al. (1970) have already observed, that *Atriplex halimus* grown on nutritive solutions with different Na^+ and K^+ concentration ratios, the best growth results are obtained when the ratio is equal to 1. It could be due to a positive biochemical interaction between these two elements, maybe relative to a kind of ionic equilibrium, inducing optimum conditions for some enzymatic systems.

In the animal physiology field, Green and Taylor (1964) point out that adenosin phosphatase (ATPase) activation depends on the occupation of two kinds of sites, one for Na^+ and the other for K^+. The maximum activation is obtained when the two sites are occupied. This supposes that the ionic equilibrium given by the synthetic medium is also found at the cellular level.

Our experiments show the Na^+ and Ca^{++} medium concentration effects on *Atriplex halimus* growth. We have no results on the cation concentration in the plant tissues. Therefore for instance, we don't know if the $[Na^+]/[Ca^{++}]$ ratio is the same in the synthetic medium as inside the plant. The absorption of each ion partially depends of the ionic gradients. Some results on *Atriplex vesicaria* and *A. spongiosa*, growing on nutritive solutions, show that the absorption of the Ca^{++} medium is modified by the K^+ and Na^+ concentrations (Osmond, 1966). In the case of another halophyte (*Suaeda maritima*), there is a competitiveness in the absorption of these two elements (Le Saos and Binet, 1987).

In our system, it seems difficult to think of an antagonism between Na^+ and Ca^{++} as the increase of the $[Na^+]/[Ca^{++}]$ ratio can induce either a stimulation or a decrease of the measured characteristics (Figures 2, 3, and 4).

A better understanding of our observations would only be possible by studying a wider range of morphogenesis and physiological parameters.

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