Hydrogen-Ion Concentration Influences upon the Rate of Mitosis in Root Tips of *Trillium sessile* L.

2. Comparative study of two buffer systems¹

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Introduction

The fact that hydrogen concentration exerts an influence upon the distribution and rate of growth among plants and animals is evidenced by the general conclusions that some organisms can tolerate a wide pH range, others only a narrow range, and still others are intermediate. Also, some organisms flourish best in an acid, others in an alkaline environment, and others find their optimum at or near neutrality. Bailey (1956) has indicated that various hydrogen-ion concentrations when adjusted with HCl and NaOH exert considerable influences upon the rate of mitosis in root tips of *Trillium sessile* L. and upon the mitotic figures within these root tips. These influences take the form of a blocking of mitosis at metaphase along with an excessive contraction of metaphase chromosomes as well as a clumping of the metaphase chromosomes. It is the purpose of this study to determine the influences of various hydrogen-ion concentrations upon the rate of mitosis in root tips of *T. sessile* L. when hydrogen-ion concentrations are adjusted with various buffering solutions.

Materials and methods

Rhizomes of *T. sessile* L. were potted in peat moss so as to provide an adequate source of fresh growing root tips. Rhizomes were rinsed in distilled water after roots had appeared, wrapped in wet paper towels to prevent excessive drying, and suspended over culture dishes containing 500 ml. of triple distilled water to which had been added 30 ml. of the buffer solutions used in this study and described by Welcher (pages 63-64 and 64-65). Roots were then placed in these solutions while still attached to the rhizomes for periods ranging from 24 hours to 96 hours. Buffer solution No. I, giving a pH range between 4.0 and 8.0, was prepared using hydrochloric acid, sodium hydroxide, boric acid, potassium chloride, potassium acid phthalate, and potassium dihydrogen phosphate. Buffer solution No. II, with the same pH range, was prepared using citric acid and disodium phosphate. All exposures were made at a temperature of 24°C, the optimum

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temperature for growth and cell division in roots of *T. sessile* L., as shown by Bailey (1954). Slides were prepared using the Feulgen technique, together with a squash method; pH determinations were made using a Beckman pH Meter, Model G.

Discussion

Table 1 shows the total percentage of dividing nuclei within root tips of *T. sessile* L. which have been exposed to various hydrogen-ion concentrations for periods of time ranging from 24 hours to 96 hours. The indicated hydrogen-ion concentrations were adjusted with buffer solution No. I, referred to above. These hydrogen-ion concentrations did not vary more than plus or minus .1 during the indicated exposure periods.

Table 1. Influence of various hydrogen-ion concentrations¹ upon the rate of mitosis in root tips of *T. sessile* L.

pH	Hours Exposure	Total % Dividing nuclei	% Prophases	% Metaphases	% Anaphases	% Telophases
4.0	24	1.8	75.0	3.6	9.9	11.4
	48	3.8	66.4	13.6	12.1	7.8
	72	1.8	66.3	6.2	17.4	10.1
	96	.7	58.2	12.7	18.2	10.9
5.0	24	4.0	72.8	1.6	14.1	11.5
	96	4.3	72.6	.3	12.7	14.4
6.0	24	3.1	75.3	.7	14.1	9.7
	96	4.3	68.4	.5	18.9	12.1
7.0	24	5.2	75.8	3.0	10.3	9.9
	48	5.0	67.7	.7	16.5	15.1
	72	2.6	67.1	.4	15.8	16.6
	96	2.2	75.1	.5	12.4	11.9
8.0	24	5.8	66.4	10.2	11.7	11.5

¹ pH adjusted with KCl, KHphthalate, KH₂PO₄, H₃BO₃, HCl and NaOH according to Welcher, CHEMICAL SOLUTIONS, pages 63-64.

Table 1 shows the total percentage of dividing nuclei within root tips of *T. sessile* L. after 24 hours exposure to a pH of 4.0 to be 1.8%. After 48 hours the percentage increased to 3.8%, however, this percentage decreased to .7% after 96 hours exposure. The percentage of prophase at a pH of 4.0 decreased from 75.0% after 24 hours to 58.2% after 96 hours. Continued exposure to this hydrogen-ion concentration produces a mild metaphase block as indicated by the figures given in Table 1, that is, 3.6% of the dividing nuclei were in metaphase after 24 hours exposure to a pH of 4.0, 13.6% after 48 hours, 6.2% after 72 hours and 12.7% after 96 hours. The percentage of anaphases increased at a pH of 4.0 upon continued exposure from 9.9% after 24 hours to 18.2% after 96 hours. The percentage of telophases did not change significantly between 24 and 96 hours exposure at this pH.

A pH of 5.0 after 24 hours shows 4.0% of the nuclei to be in some stage of mitosis and after 96 hours 4.3% of the nuclei were dividing. There were no significant change between the percentage of prophases, metaphases, anaphases, and telophases between the 24 and 96 hour periods at a pH of 5.0. The figures in Table 1 show an absence of the so-called metaphase block which was evident after continued exposure to a pH of 4.0. A mild metaphase block was also evident at a pH of 5.0 when HCl was used to make the hydrogen-ion concentration adjustments (Bailey, 1956).

After 24 hours exposure to a pH of 6.0, 3.1% of the nuclei were in some stage of mitosis, whereas after 96 hours, 4.3% of the nuclei were dividing. The percentage distribution of prophases, metaphases, anaphases, and telophases do not differ significantly between the 24 and 96 hour periods, nor do they differ significantly from the pattern of tips exposed to a pH of 5.0. A pH of 7.0 shows 5.2% of the nuclei to be in some stage of mitosis after 24 hours. This total percentage of dividing nuclei decreases so that after 96 hours 2.2% of the nuclei were in some stage of mitosis. The percentage of prophases, metaphases, anaphases, and telophases were very close to those given for a pH of 5.0 and 6.0. The only significant difference between the 24-hour exposure period and 96 hours is to be seen in metaphases. 3.0% of the dividing nuclei were in metaphase after 24 hours at a pH of 7.0, whereas only .7% were in metaphase after 48 hours. This may not be significant, but it could indicate a mild metaphase block with the root tips showing an ability to overcome this condition. A pH of 8.0 shows 5.8% of the nuclei to be dividing after 24 hours exposure. It is also evident from the figures in Table 1 that this pH of 8.0 produces a mild metaphase block in that 10.2% of the dividing nuclei were in metaphase after 24 hours. Exposure to a pH of 8.0 for periods longer than 24 hours produced death among the cells.

Table 2. Influence of various hydrogen-ion concentrations¹ upon the rate of mitosis in root tips of *T. sessile* L.

pH	Hours Exposure	Total % Dividing Nuclei	% Prophases	% Metaphases	% Anaphases	% Telophases
4	24	2.5	75.2	14.1	6.7	4.0
	48	.9	91.8	4.5	1.2	2.4
5	24	2.3	77.4	12.8	5.9	3.9
	48	1.5	80.6	17.4	.5	1.5
6	24	1.9	63.6	31.4	2.9	2.0
	48		No recognizable mitotic figures.			
7	24	3.1	76.6	20.5	1.4	1.4
	48		No recognizable mitotic figures.			
8	24	4.9	73.8	22.8	1.4	2.0
	48		No recognizable mitotic figures.			

¹ pH adjusted with citric acid and disodium phosphate buffer, given on pages 64-65 of Welcher, CHEMICAL SOLUTIONS.

When exposed to buffer solution No. I, root tips of *T. sessile* L. fail to withstand a pH below 4.0 and above 8.0 for periods of time as long as 12 hours.

Table 2 shows the influence of various hydrogen-ion concentrations upon the rate of mitosis in root tips of *T. sessile* L. when the hydrogen-ion concentration is adjusted with buffer solution No. II referred to above; that is, when citric acid and disodium phosphate are used. The same pH range is used for this buffer solution as for buffer solution No. I.

After 24 hours at a pH of 4.0 adjusted with buffer solution No. II, 2.5% of the nuclei were in some of stage mitosis. This percentage decreased to .9% after 48 hours. 14.1% of the dividing nuclei after 24 hours at a pH of 4.0 were in metaphase. The percentage of anaphases and telophases had decreased significantly after 24 hours when compared to the figures given by Bailey (1956) for tips grown in distilled water or peat moss, whereas the percentage of prophases remained approximately normal. After 48 hours at a pH of 4.0, 91.8% of the dividing nuclei were in prophase, whereas the percentage of metaphases had decreased to 4.5%. The greater portion of these prophases were very late prophases, indicating that perhaps the block is at late prophase rather than at metaphase. The percentage of anaphases and telophases had again decreased very significantly; that is, 1.2% of the dividing nuclei were in anaphase with 2.4% in telophase.

A pH of 5.0 shows a similar pattern to that for a pH of 4.0. After 24 hours at a pH of 5.0, 2.3% of the nuclei were dividing, and after 48 hours 1.5% of the nuclei were in some stage of mitosis. After 24 and 48 hours at a pH of 5.0 the metaphase block is significant; that is, 12.8% of the dividing nuclei were in metaphase after 24 hours and 17.4% after 48 hours. Also, the percentage of prophases increased between 24 and 48 hours, whereas the percentage of anaphases and telophases decreased very significantly. Only .5% of the dividing nuclei were in anaphase after 48 hours, whereas 1.5% of the dividing nuclei were in telophase.

Exposure to a pH of 4.0 and 5.0 for periods of time longer than 48 hours is lethal to root tips of *T. sessile* L., that is, when buffer solution No. II is used to make the hydrogen-ion concentration adjustments.

After 24 hours at a pH of 6.0, 1.9% of the nuclei were dividing. 31.4% of these dividing nuclei were in metaphase, indicating a significant metaphase block; 63.6% were in prophase with only 2.9% in anaphase and 2.0% in telophase. After 48 hours at a pH of 6.0 no mitotic figures were recognizable; that is, the chromosomes, etc., had undergone changes to the point of becoming indistinct, etc.

After exposure for 24 hours to a pH of 7.0 root tips of *T. sessile* L. show 3.1% of their nuclei to be in some stage of mitosis. 20.5% of these dividing nuclei were in metaphase with only 1.4% of the dividing nuclei in anaphase and 1.4% in telophase. 4.9% of the nuclei were in some stage of

mitosis after 24 hours at a pH of 8.0. Here again, we find the same general pattern; that is, 22.8% of the dividing nuclei were in metaphase with 1.4% anaphases and 2.0% telophases. Exposure for 48 hours at a pH of 7.0 and 8.0 caused death among the cells.

A closer look at the mitotic figures themselves reveals some additional influences after exposure to the two above buffer solutions. The only noticeable influence upon the mitotic figures when root tips were exposed to buffer solution No. I is an excessive contraction of metaphase chromosomes after exposure to a pH of 4.0. Also, the late prophase chromosomes seem to show some excessive contraction at this pH, however, the chromosome contraction was not extreme in either instance.

When root tips are exposed to a pH of 4.0 and a pH of 5.0 using buffer solution No. II, the only noticeable influence upon the mitotic figures was the fact that the metaphase chromosomes never contracted as much as they normally do; that is, they appeared to be considerably longer than the normal metaphase chromosome, perhaps longer by as much as one-third their usual length. A pH of 6.0 for 24 hours using buffer solution No. II produce a "stickly" condition on the part of the metaphase chromosomes, a condition similar to that shown by Bailey (1956) after exposure to a concentration of .00001 M NaCl for 24 hours. Also, the interphase nuclei were somewhat smaller and some of the mitotic figures were more or less indistinguishable at this pH. A pH of 7.0 and 8.0 using buffer solution No. II gives the same general appearance with respect to metaphase chromosomes as did a pH of 6.0. Cells exposed to a pH of 7.0 and 8.0 for 48 hours show a clumping of the metaphase chromosomes; that is, the chromosomes lost all distinctness and were present as a single mass of chromatin material.

Conclusions

1. The same hydrogen-ion concentration will give entirely different effects upon the rate of mitosis and upon the mitotic figures within root tips of *T. sessile* L. when pH adjustments are made with different buffering solutions.

2. When hydrogen-ion concentrations are adjusted with buffer solution No. I mentioned in the discussion above, root tips of *T. sessile* L. tolerate a pH between 4.0 and 7.0 for period of time as long as 96 hours. Tips fail to survive at a pH of 8.0 for periods longer than 24 hours.

3. When hydrogen-ion concentrations are adjusted with buffer solution No. II, root tips cannot withstand a pH of 4.0 and 5.0 for periods longer than 48 hours. These tips cannot tolerate a pH between 6.0 and 8.0 for longer than 24 hours.

4. Buffer solution No. I fails to show any significant effects upon the rate of mitosis except a mild metaphase block at a pH of 4.0 and a pH of 8.0.

5. Buffer solution No. II produces a very significant block at late prophase and metaphase by the end of a 24 hour exposure period. This effect is evident at all hydrogen-ion concentrations between a pH of 4.0 and a pH of 8.0.

Literature cited

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