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TP n°1

## Détermination des caractéristiques hydriques du tubercule de betterave rouge

### Introduction

Le potentiel hydrique est la résultante des différentes forces d'appel d'eau ou de surpression<sup>1</sup> et permet de rendre compte des mouvements d'eau à travers une membrane hemiperméable. Le but de ce TP est de mesurer ce potentiel hydrique et ces différentes composantes.

On admettra que:

$$\Psi_{wi} = \Psi_{si} + P + \Psi_m \quad \text{avec } \Psi_{wi} - \text{potentiel hydrique intracellulaire,}$$

$\Psi_{si}$  - potentiel osmotique intracellulaire

P - surpression hydrostatique et

$\Psi_m$  - potentiel matriciel

### Les techniques utilisées

#### Mesure du potentiel hydrique: détermination des variations de poids

Les 14 tranches semblables de betterave sont découpées sur du papier aluminium, chaque tranche est biseautée à une extrémité de façon de réduire le contact avec les parois du tube ce qui pourrait fausser les mesures.

Ensuite on prépare 11 solutions de saccharose à partir de la solution "mère", molaire (voir tableau ci-dessous).

Concentration de saccharose (en M)	0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1	1,1	1,2	1,5
ml de H2O distillée	30	27	24	21	18	15	12	9	6	3	0	∞	∞	∞
ml se saccharose (1M)	0	3	6	9	12	15	18	21	24	27	30	∞	∞	∞

Le poids des tranches de la betterave est mesuré puis elles sont plongées dans les solutions de saccharose pendant 1h 30. On agite les tubes toutes 15 minutes. Après 1h 30 les tranches sont à nouveau pesées.

Précautions à prendre: Afin d'éviter l'évaporation de l'eau on évite de laisser les morceaux de betterave à l'air libre en les gardant enveloppés de papier aluminium.

Les résultats de cette expérience sont présentés dans le tableau suivant:

<sup>1</sup> La pression étant la force par unité de surface

concentration de saccharose (en M)	0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1	1,1	1,2	1,5
ml de H2O distillée	30	27	24	21	18	15	12	9	6	3	0	∞	∞	∞
ml se saccharose (1M)	0	3	6	9	12	15	18	21	24	27	30	∞	∞	∞
poids initial P <sub>0</sub> (en g)	6,5191	5,5550	5,2398	6,5940	5,6210	5,6029	6,0244	6,1756	5,5749	5,2837	4,6001	5,8138	6,5795	6,8935
poids final P (en g)	7,4824	6,5856	5,9420	7,2973	6,2649	6,1876	6,2756	6,1339	5,3303	6,0026	4,1953	4,7690	5,7763	4,9253
$\sqrt[3]{(\Delta p/p_0)}$	0,529	0,570	0,512	0,474	0,486	0,471	0,347	-0,189	-0,353	0,514	-0,445	-0,564	-0,496	-0,658

note: Le résultat obtenu pour C<sub>0</sub>=0,9 M est faux. Il s'agit probablement d'une erreur de pipettage. Nous n'allons pas tenir compte de cette mesure.

A partir de ces calculs nous avons tracé le graphique montrant la variation du  $\Delta P$  en fonction de la concentration du milieu extracellulaire. En effet, nous avons choisi de représenter la fonction  $f(C_s) = \sqrt[3]{[(\text{Poids final} - \text{Poids initial}) / \text{Poids initial}]}$  car à l'aide de cet artifice de calcul on accentue les variations de la courbe, il est ainsi plus facile de repérer les points importants.

### Interprétation des résultats

Lorsque deux solutions, dont l'osmolarité est différente, sont séparées par une membrane hemiperméable il s'établit un flux d'eau de façon qu'à l'équilibre on a:

$$\Psi_{wi} = \Psi_{wo} = \Psi_{so}$$

A l'équilibre le potentiel hydrique intracellulaire s'égalise avec le potentiel hydrique du milieu extracellulaire.

Lorsque le potentiel hydrique du milieu extracellulaire (dont la concentration en saccharose est connue) est égal au potentiel hydrique intracellulaire le flux total d'eau est nul. Alors le poids du fragment du tissu ne varie pas.

Regardons le graphique de la variation du poids des fragments de betterave. Nous avons obtenu une courbe sigmoïde décroissante. On constate que pour C<sub>0</sub> # 0.65 M <sup>(2)</sup> la variation du poids est nulle. Pour cette valeur on a  $\Psi_{wiN} = -2.0 \text{ MPa}$ . On utilise la courbe d'étalonnage de la page 2-19 du polycopié pour obtenir la valeur en MPa à partir de la concentration osmolaire. Cette valeur correspond donc au potentiel hydrique physiologique; la cellule n'a ni gagné ni perdu de l'eau.

Lorsque  $0 < C_s < 0.65 \text{ M}$ , à t<sub>0</sub>  $\Psi_{wi} < \Psi_{wo}$  donc il y a une entrée d'eau dans la cellule. A t<sub>équilb</sub> on a  $\Psi_{wi} = \Psi_{si} + P = \Psi_{wo} = \Psi_{so}$ . Ici on néglige  $\Psi_m$ . La pression hydrostatique ou la pression de turgescence (P) augmente à mesure que la concentration du saccharose diminue. Pour l'eau pure on a  $\Psi_{si} = P$  car  $\Psi_{wi} = \Psi_{wo} = \Psi_{so} = 0$ .

Pour  $0.66 \text{ M} < C_s$ ,  $\Psi_{wi} > \Psi_{wo}$ , il y a donc une sortie d'eau de la cellule. La vacuole diminue du volume, le cytoplasme se détache de la parois. La cellule est plasmolysée. Lorsque la concentration de saccharose est telle que V<sub>cellulaire</sub> reste constant et la pression de turgescence est nulle, on parle de la "plasmolyse limite".

<sup>2</sup> Pour le saccharose 1M  $\Leftrightarrow$  osmole

## Mesure du potentiel osmotique - *détermination de l'abaissement du point de congélation d'un extrait de betterave ( cryoscopie )*

On utilisera la relation découverte par RAOULT:  $\Delta T = 1.86 \sum C_s$

où  $C_s$  - concentration osmolaire s'exprimant en osmoles

La présence d'un soluté abaisse le point de congélation de la solution. Ainsi en mesurant la différence entre les points de congélation de la solution et du solvant pur, on peut calculer la concentration osmolaire de la solution.

On mesure donc d'abord le point de congélation d'eau distillée, le "zéro du commerce" n'étant pas suffisamment précis.

Ensuite nous déterminons le point de congélation du suc cellulaire. Toutes les mesures sont répétées trois fois, les calculs sont faits à partir des moyennes des mesures.

Les résultats:

	H2O		suc cellulaire		y
T01	0,08		Ts1 -1,52		Ty1 -1,16
T02	0,08		Ts2 -1,53		Ty2 -1,18
T03	0,06		Ts3 -1,58		Ty3 -1,16
moyenne	0,073		-1,543		-1,17
Cs= 0,869	les concentrations sont en M				Cy= 0,668

### L'interprétation des résultats

La cryoscopie nous permet de mesurer l'osmolarité du suc cellulaire. On a obtenu  $C_s = 0.869$  osmoles, ce qui correspond à un  $\Psi_{sIN} = -2.8$  MPa.

Comme on a dit précédemment:  $\Psi_{wIN} = \Psi_{sIN} + P_N + \Psi_m$ . On a donc  $P_N + \Psi_m = 0.8$  MPa.

Les résultats obtenus par la technique d'observation microscopique de la plasmolyse limite ( résultats empruntés à Lionel Galliberd et Cécilie ~~xxxxxxxx~~ ) donnent

$\Psi_{sIN} = -3.25$  Mpa. Dans la cellule d'autres forces d'appel d'eau peuvent intervenir ( présence de mucilages ). La technique d'observation microscopique de la plasmolyse limite ne permet pas de discerner ces différentes forces. Le résultat obtenu correspond en fait à  $\Psi_{sIN} + \Psi_m$ .

Comme  $\Psi_m$  est une force d'appel d'eau, sa valeur numérique est négative. C'est la raison pour la quelle les mesures de  $\Psi_{si}$  par la cryoscopie ( mesure réellement la concentration en osmotocums du milieu intracellulaire) donne systématiquement des valeurs moins négatives que celles obtenus par la méthode de la plasmolyse limite.

Nous avons donc  $\Psi_{sIN} + \Psi_m = -3.25$  MPa et  $\Psi_{sIN} = -2.8$  MPa. D'o?  $\Psi_m = -0.45$  MPa.

Or  $P_N + \Psi_m = 0.8$  MPa d'o?  $P_N = 1.25$  MPa.

### Conclusion

A l'aide de ces expériences on a pu mesurer les différentes composantes du potentiel hydrique dans les conditions physiologiques. On a aussi pu observer le comportement d'une cellule lorsque la composition de son milieu extracellulaire varie.

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