EFFECT OF RAFFINOSE FAMILY OLIGOSACCHARIDES ON SEED GERMINATION OF WILD AND MUTANT TYPES OF CHICKPEA (*CICER ARIETINUM*)

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ABSTRACT

The raffinose family oligosaccharides (RFOs) are a group of soluble galactosyl derivatives which include raffinose and stachyose thought to play a number of roles in plant development. They are providing a source of rapidly metabolizable carbohydrate during germination. In contrast to their potential for promoting germination, RFOs represent anti-nutritional units for monogastric animals when consumed as a component of feed. The exact role of RFOs during seed development and germination has not been experimentally determined, but it has been hypothesized that RFOs are required for successful germination. To serve as an energy sources, RFO's must be degraded into their component monosaccharides. However, low RFO had not significantly delayed or reduced in chickpea seed germination. We conclude that RFOs are not an essential source of energy during chickpea seed germination in chickpea seeds.

Key Words: Chickpea, carbohydrate, Raffinose family oligosaccharides, seed germination

I. INTRODUCTION

The exact function of raffinose family oligosaccharides (RFOs; raffinose, stachyose, and verbascose) in germinating seeds is largely unknown, but it is clear that seeds require a large amount of energy during germination (**Bewley and Black, 1994**). This energy is hypothesized to come from stored carbohydrates; sucrose and RFOs are the most abundant of the soluble sugars (**Peterbauer and Richter, 2001**) but account for only a small portion of the total carbohydrates present in the seeds (**Ziegler, 1995**). Since the breakdown of seed components during germination has been studied extensively, it is known that protein, oils and polymeric carbohydrates each break down slowly over a period of several days (**Bewley and Black, 1994**). This period extends beyond germination and thus cannot be solely responsible for fulfilling the energy demands of the germinating seed. Because of the implication that RFOs play critical roles in seed development and germination, the objective of this article is to determine the survival potential of chickpea lines with low RFOs as compared to sibling lines with high and wild-type RFO content.

II. MATERIALS AND METHODS

2.1 Plant material

The experiment was carried out to study the survival percentage of the chickpea accessions received from ICRISAT,Hyderabad. The experimental material comprising of 215 genotypes including check varieties of chickpea were evaluated in randomized block design with three replications at Department of Pulses, Research farm, Coimbatore, Tamil Nadu during Rabi 2009. Each genotype was grown in single row of 3 meter length with row to row and plant to plant distance maintain as 30cm and 10cm. The recommended package and practices were followed to raise the healthy crop. The seeds from 215 accessions were phenotyped for seed oligosaccharide content using high performance liquid chromatography (HPLC). Five lines were selected with wild-type RFO phenotypes and five lines with low RFO phenotypes. The germination and survival percentage of 215 chickpea accessions were recorded.

2.2 Results and Discussion

Chick pea plants exhibiting high wild or low RFO phenotypes were grown in favourable environment. Five lines were selected with wild-type RFO profiles and five lines were selected with low RFO profiles (Table1) based on HPLC analysis. The survival of plants was recorded 15 days after sowing in the extreme types. The mean survival percentage in the ten extreme accessions of chickpea was ranged from 4% to 76%, with a mean value of 32.80%. The lowest survival percentage was recorded by ICC6263 (4%) with total RFO value of 17.67 mg g^{-1} and the highest survival percentage was recorded by ICC4593 (76%) with the total RFO value of 102.78 mg g^{-1}.

The survival percentage of the chickpea genotypes not varies depending on the raffinose content but it depends upon overall RFO content and specifically to stachyose. Stachyose was the major compound found in chickpea quantified by HPLC. Germination was monitored for the lines with contrasting RFO profiles to determine if reduced RFOs would have a negative impact on standard germination. There were a significant differences for standard germination between wild-type and low RFO lines.

The extremes of survival was monitored with total RFO content. Chickpea seeds with low RFOs exhibited less survival percentage than the wild types with more RFO content. The high and wild-type RFO phenotypes exhibited a significant survival in chickpea seeds but it was reported that there is a drastic reduction in survival in pea seeds (**Blochl *et al.*, 2007**). Chickpea seeds appeared to have less germination efficiency with low RFO content (**Fig.1.**). Seeds depend on degradation of stored carbon reserves for energy during germination. These reserves are mostly in the form of starch, soluble sugars, oil and proteins. In addition, stored RFO may play an important and essential role in the early stages of germination (**Downie and Bewley 2000; Blöchl *et al.*, 2007**) when the breakdown of other stored reserves are unable to meet the energy demand (**Bewley and Black 1994**). A reduction in RFO concentration upon germination is seen in legume seeds such as cow pea, jack bean, and soybean (**Martin-Cabrejas *et al.*, 2008**), where RFO degradation is almost complete before radicle protrusion (**Horbowicz *et al.*, 1998; Lin *et al.*, 1998; Modi *et al.*, 2000**). A role for RFO in germination is also supported by studies where inhibition of α-galactosidase activity by 1-deoxygalactonojirimycin (DGJ) significantly delays seed germination (**Blöchl *et al*., 2007**). This inhibitory effect of DGJ is reversed by galactose application or partially by sucrose suggesting that galactose plays an important role in the sugar signalling pathway (**Blöchl *et al*., 2007**). In contrast to previous reports, **Dierking and Bilyeu (2009)** recently reported that water imbibition and germination rate of soybean seeds with normal or low RFO concentrations is not significantly different. Nor does a difference in raffinose and stachyose concentrations affect germination rate and emergence time in soybean seeds (**Neus *et al*., 2005**). Similarly, maize seed germination is not affected by the absence of raffinose (**Brenac *et al*., 1997**). These studies suggest that utilization of RFO in the initial germination processes may not be absolutely required for germination. This result is in contrast with germination in pea seeds where the addition of sucrose and galactose partially overcame the reduced germination effect of DGJ on germinating seeds (**Blochl *et al*., 2007**).

Overall, the results of this study indicate that wild-type levels of total RFO and stachyose are required for efficient germination of chickpea seeds. The suggested necessity of RFO mobilization for efficient seed germination is shown here to be on par, for chickpea. There was a significant difference between germination of wild-type RFO and low RFO chickpea. Our results demonstrate that chickpea and soybean have a fundamental difference in oligosaccharide requirements during germination (**Dierking and Bilyeu, 2009**) but there is a similarity between pea and chickpea. This data resembles the long held assumption that accumulated seed RFOs are required for successful chickpea seed germination. We have shown that reducing raffinose and stachyose in chickpea seeds to very minimum level, without selection for agronomic characteristics, reduce or delay chickpea seed germination. So efforts have to be taken to reduce the RFO level to a considerable amount not less 50% provides additional incentive for chickpea breeding programs to incorporate the low RFO trait into elite varieties to increase the value of chickpea used for consumption by humans and monogastric animals.

REFERENCES

1. Bewley,J.D. and Black, M., 1994, Seeds: Physiology of development and germination. New York: Plenum Press.
2. Blochl A., Peterbauer,T., and Richter, A., 2007, Inhibition of raffinose oligosaccharide break down delays germination of pea seeds. J Plant Physiol., 164:1093–6.
3. Brenac, P., Horbowicz, M., Downer, S. M., Dickerman, A. M., Smith, M. E. and Obendorf, R. L., 1997, Raffinose accumulation related to desiccation tolerance during maize (Zea mays L.) seed development and maturation. J. Plant Physiol., 150: 481-488.
4. Dierking, E. C. and. Bilyeu, K. D., 2009,Raffinose and stachyose metabolism are not required for efficient soybean seed germination. J. Plant Physiol., 166: 1329-1325.
5. Downie, B. and Bewley, J. D., 2000, Soluble sugar content of white spruce (Picea glauca) seeds during and after germination. Physiol. Plant., 110:1-12.
6. Horbowicz, M., Brenac, P., and Obendorf, R.L., 1998, Fagopyritol B1, O-α -D-galactopyranosyl-(1→2)-D-chiro-inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. Planta., 205: 1-11.
7. Lin, T. P., Yen, W. L. and Chien, C. T., 1998, Disappearance of desiccation tolerance of imbibed crop seeds is not associated with the decline of oligosaccharides. J. Exp. Bot., 49: 1203-1212.

8. Martin-Cabrejas, M. A., Diaz, M. F.,.Aguilera, Y., Benitez, V., Molla, E., and Esteban, R. M.,2008, Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. Food Chem., 107: 1045-1052.
9. Modi, A. T., McDonald, M. B. and Streeter, J. G.,2000, Soluble carbohydrates in soybean seeds during development and imbibition. Seed Sci. and Technol., 28: 115-127.
10. Neus, J. D., Fehr, W. R. and Schnebly, S. R., 2005,Agronomic and seed characteristics of soybean with reduced raffinose and stachyose. Crop Sci., 45: 589-592.
11. Peterbauer, T. and Richter, A., 2001, Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Sci. Res., 11: 185-197.
12. Ziegler, P. 1995, Seed development and germination. New York: Marcel Dekker Inc.

Table1. Effect of total RFO on survival percentage of chickpea seeds

S. No.	Accession Number	Survival percentage (%)	Raffinose (mg/g)	Ciceritol (mg/g)	Stachyose (mg/g)	Total RFO (mg/g)
Low RFO types						
1	ICC13816	8	0.16	9.03	2.77	11.96
2	ICC2072	12	2.3	27.34	2.93	32.57
3	ICC12968	12	3.41	4.36	3.17	10.94
4	ICC9895	8	3.01	14.9	7.37	25.28
5	ICC6263	4	0.16	10.06	7.45	17.67
	Mean	**8.80**	**1.81**	**13.14**	**4.74**	**19.68**
Wild RFO types						
6	ICC1194	52	5.23	52.38	55.78	113.39
7	ICC10393	40	3.72	31.06	57.61	92.39
8	ICC14098	72	5.86	48.89	57.61	112.36
9	ICC1205	44	6.1	38.67	57.69	102.46
10	ICC4593	76	2.3	41.05	59.43	102.78
Mean		**56.8**	**4.642**	**42.41**	**57.624**	**104.676**
Overall mean		**32.80**	**3.23**	**27.77**	**31.18**	**62.18**

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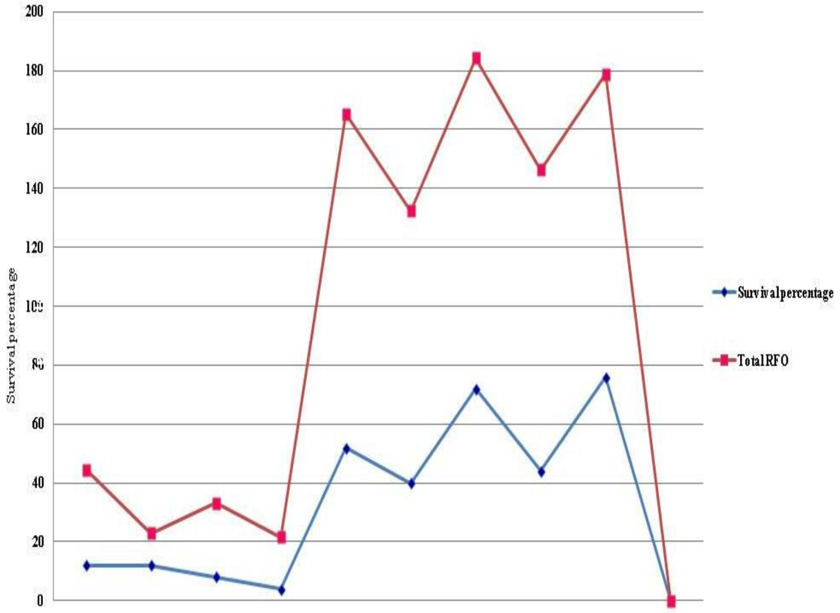

Fig.1. Effect Of RFO In Chickpea Seed For Survival of The Seedlings