Original Article

TETRAZOLIUM TEST TO EVALUATE THE VIABILITY OF OIL RADISH SEEDS

TESTE DE TETRAZÓLIO PARA AVALIAÇÃO DA VIABILIDADE DE SEMENTES DE NABO FORRAGEIRO

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ABSTRACT: The use of tetrazolium test is important in the evaluation of seeds lot quality and it has been adopted for vigor and viability identification for several species. The interest on the production of oil radish is increasing since the seeds were considered a good source of oil for biofuel production. The development of the tetrazolium test methodology for seeds of this species can improve the seed quality control process, and additionally will provide information for the characterization of remaining seeds (dead or dormant) in the germination tests. To verify the ideal conditions to tetrazolium test was conducted two experiments. At the first, oil radish seeds cultivar CATI AL-1000, lots from 2001 and 2006 were submitted to imbibition between paper in water for 6 hours. After the longitudinal cut in the longest direction, the seeds were immersed in the tetrazolium solution at the concentrations of 0,075%, 0,5% and 1% at 25°C for 3h, 12h and 18h. In the second experiment, oil radish seeds cultivar CATI AL-1000 lot from 2005 and IPR 116 cultivar, lots from 2004 and 2005 were immersed in the concentrations of ,1%; 0,2%; 0,3% and 0,4% of tetrazolium solution for 12 hours at a 25°C, 30°C, 35°C and 40°C. At the first experiment was observed the necessity of test intermediate concentrations between 0,075% and 0,5%, since with 0,075% the seeds stained weakly and with 0,5% the test results, were overestimated. In the second experiment was observed that the 0,3% concentration at 30°C can be recommended for the utilization of tetrazolium test to evaluation of oil radish seeds viability.

KEYWORDS: *Raphanus sativus*. Pre-condition. Seed quality.

INTRODUCTION

The species *Raphanus sativus* L. is a plant from the Brassicaceae family with a relatively easy cultivation by the natural hardiness and tolerance to the adverse conditions besides the ability to develop relatively well in soils with low fertility and slightly acids.

Is a plant very used in the crop rotation and green manure due to the higher potential of nutrients cycling. Besides this, the roots promotes important physical effect in the soil allowing a biological prepare and avoiding the loosening of the soil (CRUSCIOL et al., 2005). The seeds have significant content of oil with a relative easy extraction, what can becomes important to the rural producers for the production of oil in the Biodiesel Program (SILVA, 2007). The potential for the oil extraction until today, it was not much explored, both by the low technology for the production of grains or by the availability of quality seeds.

A big challenge for the research institutions and companies producers of seeds, has been the evaluation of lots viability, what allows the selection of seeds on sowing. For species like *R. sativus* L., there are not enough researches in this area.

The knowledge about the quality of seeds lots depends of the availability of correct methodologies, what takes to the obtainment of trust results (MCDONALD, 1998). The tetrazolium test has been used with success in the quality programs control of seeds, to be a fast method who estimates the viability of lots of seeds (HAMPTON and COOLBEAR, 1990). Beside this, this test can supplementary the germination test results of the lots with dormant seeds and diagnose deterioration causes (KRZYZANOWSKI et al., 1999) in a short period of time (MENDONÇA et al., 2001).

The efficiency of tetrazolium test in evaluate the viability of seeds depends of the development of adapted methods for each specie in order to define the appropriate conditions for the hydration, prepare, coloration and the evaluation of seeds. This test is based on the activity of enzymes dehydrogenates in the respiratory process of tissues. During the respiration, occurs the liberation of hydrogen ions in which the 2,3,5 triphenyl tetrazolium chloride salt reacts, forming a substance with red color and insoluble, named formazan in the alive tissues of seeds (DELOUCHE et al., 1976). The velocity in which the tetrazolium salt is absorbed by the seeds tissues, depends of the number of physical obstacles in the seeds, like the

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coat (PINÃ RODRIGUES; SANTOS, 1988). In many species, the pre-conditioning of seeds is necessary, aiming to the activation of respiratory system and the penetration of the solution (VIEIRA; VON PINHO, 1999).

Besides pre-conditioning, factors like the concentration of solution or even the time of coloration in the solution, can affect the efficiency of test in the evaluation of seed quality. The period necessary to the development of adequate coloration according to Krzyzanowski et al. (1999), varies with the specie staying between 30 and 240 minutes.

Some researches has been done trying to adjust the methodologies for the use of tetrazolium test in many species, like squash (Dias et al., 2001) and summer squash (Barros et al., 2005). However, the utilization of the test is restricted, mainly to those seeds of some species like soybean (FRANÇA NETO et al., 1998, 1999), corn (DIAS; BARROS, 1995, 1999), forages (CARVALHO; TOLEDO, 1976; DIAS; ALVES, 2001), beans (SANTOS et al. 2007), cotton (VIEIRA; VON PINHO, 1999) and peanut (BITTENCOURT; VIEIRA, 1999). For viability evaluation of oil radish seeds by the tetrazolium test there are no methodologies. Based on this, the objective of this work was to provide subsidies to the adequacy of methodology for the realization of tetrazolium test in oil radish seeds.

MATERIAL AND METHODS

The research was divided in two experiments: in the experiment one were used two lots of oil radish seeds, cultivar CATI AL-1000, lot 1 and lot 2, from the region of Varginha, MG. The seeds were stored in the cool chamber. The moisture content of seeds was determined for the oven method in *105°C* per 24 hours (BRASIL, 2009), with two replications of 10g of seeds.

For the realization of germination test, the sowing was realized in gerbox box on sand substrate washed and sterilized. Then, the box was transferred to the BOD with alternate temperature of 20-30°C with 12 hours of photoperiod. Were used four replications with 50 seeds each, and the results were expressed in percentage of normal seedlings at the third day (first count), and seventh day (final count), computing the percentage of normal seedlings, abnormal infected, abnormal deformed and dead seeds. The germination speed index was calculated according Maguire (1962), computing the number of seeds with at least 1mm of radicle protrusion.

The emergency was realized in soil and sand substrate in the proportion of 1:2 in plastic trays. The moisture of substrate was adjusted to 60% of capacity of retention. After sowing four replications of 50 seeds, the trays were maintained at 20ºC in growing chamber. From the beginning of emergency were realized evaluations daily, computing the initial stand at the third day and the number of emerged seedlings until the stabilization of stand. The emergency speed index was determinate according the proposal of Maguire (1962).

For the tetrazolium test, were realized pre-tests for the time of pre-conditioning of seeds between papers moistened with 2,5 times the weight of paper per 6 hours, 12 hours and 24 hours, testing too, the moistened of intact seeds, with total remove of coat, seeds with cuts in the distal region to the embryonic axis and longitudinal cut in the higher direction with just one half of seed with the embryonic axis used. After decided about the conditioning of 6 hours with longitudinal cut in the higher direction, the seeds were totally submerged in tetrazolium solution (pH 6,5) in the concentrations of 0,075%; 0,5% and 1,0% and maintained in the dark at 25°C in BOD per 3 hours, 12 hours and 18 hours. Were used four replications of 50 seeds for each treatment.

After the development of coloration, the half of seeds were washed in current water and left submerged in water until the evaluation moment in refrigerator up to 12 hours. Later, were examined and according to the extension, intensity of red tones, appearance of white areas, aspect of tissues and localization of these colors in relation to the essential areas of growth, the seeds were classified in the categories of viable and nonviable (Figure 1).

A completely randomized design was used in a factorial 2x3x3 scheme (2 lots, 3 concentrations of tetrazolium solution and 3 periods of imbibitions in the tetrazolium).

For the experiment 2 were used two lots of oil radish seeds cultivar IPR 116, lot 3 produced in the crop of 2004 and lot 4 from the crop of 2005; and one lot of CATI – AL 1000 cultivar crop of 2005, lot 5. The seeds were stored in cold chamber. The characterization of profile of lots, the determination of moisture content, germination, first count, germination speed index, emergency, initial stand and emergency speed index were realized according described for the experiment 1. For the tetrazolium test, the seeds were pre-conditioning between papers moistened with 2,5 times the weight of paper per 6 hours, following the longitudinal cut, being that just one half of seeds with embryonic axis were used. The seeds were totally submerged in tetrazolium solution (pH 6,5) in the concentrations of 0,1%, 0,2%, 0,3% and 0,4% and maintained in

the dark at temperatures of 25°C, 30°C, 35°C and 40°C per 12 hours. Were used four replications of 50 seeds for each treatment.

Figure 1. Categories of seeds founded in the tetrazoilum test in lots of oil radish seeds. Category A (viable)- embryo with pink coloration or less than 50% of the embryo without color without reach the embryonic axis and tissue with a normal and firm appearance. Category B (non-viable), B1- more than 50% of cotyledons without color; B2- region of embryonic axis without color; B3- embryo with a red carmine coloration and B4- embryo completely without color.

After the development of coloration, the same procedure realized in the experiment 1, was done in the experiment 2. Was used a completely randomized design in a factorial 3x4x1 scheme (3 lots, 4 concentrations of tetrazolium solution and 1 period of imbibition in the tetrazolium).

For both experiment, the dates were previously submitted to the testes of normality of residues and homoscedasticity of variances. The dates of count were submitted to the variance analyses and the means compared one each other for the Scott-knott test, with 5% of probability and transformed in . The statistical analyses were realized with the help of statistical program SISVAR® (FERREIRA, 2000).

RESULTS AND DISCUSSION

Experiment 1: $\sqrt{X}$

The moisture content of lots had the average of 6,5% in the occasion of realization of the test, for lot 1 of 6,0% e lot 2 of 7,0%, what indicates uniformity of lots in relation to this factor what can interfere in the pre-conditioning period. The percentual of germination, first count of germination and the germination speed index of lot 2 was higher than lot 1, being in this last, observed a higher percentage of abnormal infected seedlings and dead seeds.

In the Table 1, can be visualized the dates related to the vigor of lots, evaluated by the initial stand, emergency and emergency speed index. It was observed in the lot 2, superior results of vigor in relation to the lot 1.

In a previous test of tetrazolium test, it was observed that the seeds moistened per 24 hours, germinated during the pre-conditioning. Already the seeds in pre-conditioning per 12 hours, presented their coat with softening, easily falling off and injuring the embryonic axis of seeds in the moment of cut, being only the period of 6 hours in water the time which permits the cut of seeds with more easily.

It is important to emphasize the necessity of longitudinal cut of seeds in the higher direction before the immersion in the tetrazolium solution, cause the presence of coat becomes hard the penetration of solution conferring to the seeds a desuniformity coloration or a no coloration. With the total removal of coat without the longitudinal

cut, the seeds were injured, what hid the results of test. Similar results verifying the necessity of remove coat of seeds of Brassicacea family were obtained per Debeaujon et al. (2000) for *Arabidopsis* seeds.

Table 1. Results of normal seedlings in the initial stand–IS (%); final stand–FS (%); emergency speed index-ESI in the emergency test of oil radish seeds. Results of normal seedlings in the first count – FC (%); germination– G (%); infected abnormal seedlings–IA (%); dead seeds– M (%) and germination speed index- GSI obtained of 2 lots of oil radish seeds.

Lots	Emergency Tests		
	IS	FS	ESI
1	0 b	20 b	7,74 b
2	49 a	75 a	18,48 a

Lots	Tests				
	FC	G	IA	D	GSI
1	1 b	28 b	27 a	44 a	6,62 b
2	37 a	87 a	10 b	3 b	43,38 a

Mean values followed by same tiny letter in the column not differ at F-test.

There was a significant interaction between the lots, concentrations and times in the tetrazolium solution (Table 2). The immersion of seeds in the tetrazolium solution per 3 hours not allowed difference between lots of oil radish. Already with 12 hours, the difference between lots was observed for all concentrations. In the period of 18 hours it was possible to observe the separation of lots, except in the concentration of 0,5%. It is important to observe that with 18 hours, the seeds had their embryonic axis elongate, difficulting the observation of possible injuries in the embryonic axis and not allowing separate seeds in different categories.

Table 2. Percentage of oil radish viable seeds obtained by the tetrazolium test in function of the solutions concentrations (%) and the time in the tetrazolium solution (hours).

Lots	Concentrations (%)	Solution time (hours)		
		3	12	18
1	0,075	2 a C	21 b B	40 b A
2		2 a B	91 a A	90 a A
1	0,5	74 a B	78 b B	86 a A
2		83 a A	92 a A	90 a A
1	1,0	79 a A	74 b A	74 b A
2		80 a A	87 a A	89 a A
CV(%)		7,13		

Mean values followed by same tiny letter in the column and capital letter in the line does not differ at 5% level of probability.

The results observed in the concentration of 0,075% of solution per 12 hours coincides with the germination results (Table 1). However in this concentration it was difficult to distinguish categories, cause the weak coloration of the seeds. In this same period of 12 hours, the concentrations of 0,5% and 1,0% had their values super estimated. In the concentration of 1,0% was observed a coloration more intense of seeds difficulting the classification of the seeds.

The concentration of 0,075% is also recommended to other oleaginous seeds like cotton (SANTOS et al., 1992) and *Lafoensia pacari* (MENDONÇA et al., 2006), *Butia cap*itata (FERNANDES et al., 2007), and beans feijão (MUASYA et al., 2002; 2006) besides *Brassica rapa, Hordeum vulgare, Brassica napus, Avena sativa, Pastinaca sativa* and *Pisum sativum* (POULSEN et al., 2006).

It is observed in general, that the tetrazolium test super estimated the results of viability of seeds, being the results of tetrazolium test and germination test, similars. This results however, can present considerable differences due the possible infestations with pathogens in the lot. In this way, not all the abnormalities found in the seedlings can be observed in the embryo and, like consequence, the tetrazolium test can show higher results.

The choose of the adequate methodology to the use of tetrazolium test should be based in the facility for differentiation in viable and non viable tissues and in the capacity of differentiate lots with distinct physiological quality. Another fact that must be considered in the evaluation of viability of seeds is the time of test execution, because according with Piana et al. (1992) and França Neto et al. (1998), the speed in the evaluation provides advantages like the possibility of disposing of lots with inadequate quality and prevents the commercialization of lots of low physiological quality seeds. For oil radish seeds, the germination test had duration of 7 days, while the results of tetrazolium test were obtained in just one day.

For the indication of ideal methodology of tetrazolium test to oil radish seeds, exists yet, based on results of the experiment 1, the necessity of refinement of the test involving the higher number of lots and adjustment of the concentrations of tetrazolium solutions, being this, proposed in the experiment 2.

For the indication of ideal methodology of tetrazolium test to oil radish seeds, exists yet, based on results in the experiment 1, necessity of refinement of the test involving the higher number of lots, a adjust too, the concentrations of tetrazolium solution, being this, proposed in the experiment 2.

Experiment 2:

The moisture content of lots had the average of 7,5%. The percentual of germination, first count of germination and the germination speed index of lot 5 was higher than the lots 3 and 4 (Table 3), being observed in the lot 3, higher percentage of abnormal infected seedlings and dead seeds. The lot 4 have their values intermediate to the lots 3 and 5.

Table 3. Results of normal seedlings in the first count – FC (%); germination– G (%); infected abnormal seedlings–IA (%); dead seeds– M (%) and germination speed index- GSI obtained of 3 lots of oil radish seeds. Results of normal seedlings in the initial stand–IS (%); final stand–FS (%); emergency speed index- ESI in the emergency test of oil radish seeds for three lots

Lots	Emergency Tests		
	IS	FS	ESI
3	13 b	29 c	3,75 c
4	11 b	45 b	6,19 b
5	21 a	84 a	11,83 a
CV (%)	5,30	6,00	4,20

Lots	Tests				
	FC	G	IA	D	GSI
3	2 c	28 c	19 a	53 a	3,70 c
4	22 b	50 b	14 a	36 b	6,58 b
5	42 a	89 a	7 b	4 c	12,20 a
CV (%)	2,00	3,50	14,00	17,68	10,50

Mean values followed by same tiny letter in the column and capital letter in the line does not differ at 5% level of probability.

In Table 3, can be visualized the dates related to the vigor of lots, evaluated by the initial stand, emergency and emergency speed index. In this test, it was observed for the lot 5, higher results in the vigor in relation to the lots 3 and 4, being again, the lot 3 the lot with lower values and the lot 4 with intermediate values.

Works has been demonstrating the limitation around tetrazolium test. One of these limitations is the correct interpretation of results of the test, mainly cause the difference of intensity of the seeds coloration which is variable between the species (OLIVEIRA et al., 2009). These differences can cause interpretation problems during the evaluation of seeds. For this reason it is interesting the use of standardized methodologies, correlating the observed colors in the seeds with a reference non subjective.

In this study, was observed in the tetrazolium test that the 0,1% concentration of solution, colored the seeds weakly, what difficult the visualization possibly due to the combination of short time of exposure and lower concentration of solution. For 0,4% concentration, the seeds colored strongly with a red carmine color very intense, characteristic of a tissue in intense respiratory

activity (deterioration process), what difficult the separation in viable and non viable seeds.

The concentration of 0,2% in the tested temperature had the values of viable seeds sub-estimated for the lots 2 and 3, approaching just for the lot 1 when compared with the results of germination and vigor tests, like emergency speed index

The concentration of 0,3% colored the seeds with a pink tone, what facilitated the separation between viable and non viable seeds considering that this was the best concentration tested, mainly for the temperatures of 30ºC and 35ºC.

The values of viable seeds obtained with the 0,3% concentration at 30°C are compatible and complementary when related with the germination and vigor tests for all lots. All the concentrations at 40°C, colored strongly the seeds, what difficult the evaluation of this seeds (Table 4 and 5).

Table 4. Percentage of oil radish viable seeds obtained by the tetrazolium test in function of the lot, temperature (°C) and solution concentrations (%).

Lots	Temperature	Solution Concentration			
		0,1	**0,2**	**0,3**	**0,4**
3	25	27 Cc	27 Cc	49 Bb	59 Aa
4		34 Bb	37 Bb	49 Ba	51 Ba
5		39 Ad	49 Ac	66 Ab	56 Aa
3	30	29 Ba	30 Ca	27 Ca	29 Ca
4		31 Bc	35 Bb	48 Ba	48 Ba
5		44 Ad	49 Ac	77 Aa	63 Ab
3	35	37 Ba	41 Ba	29 Cb	40 Ca
4		38 Bd	43 Bc	48 Bb	57 Ba
5		52 Ab	54 Ab	72 Aa	72 Aa
3	40	44 Cb	49 Ca	45 Cb	51 Ba
4		50 Bc	53 Bb	58 Ba	59 Aa
5		68 Ab	58 Ac	81 Aa	57 Ac
CV(%)		5,03			

Mean values followed by same tiny letter in the column and capital letter in the line does not differ at 5% level of probability.

Table 5. Percentage of oil radish viable seeds obtained by the tetrazolium test in function of the temperature (°C), lot and solution concentrations (%).

Lots	Temperature	Solution Concentration			
		0,1	**0,2**	**0,3**	**0,4**
3	25	27 C	27 C	49 A	59 A
	30	29 C	30 C	27 C	29 D
	35	37 B	41 B	29 C	40 C
	40	44 A	49 A	45 B	51 B
4	25	34 C	37 C	49 B	51 B
	30	31 C	35 C	48 B	48 B
	35	38 B	43 B	48 B	57 A
	40	50 A	53 A	58 A	59 A
5	25	39 D	49 C	66 D	56 C
	30	44 C	49 C	77 B	63 B
	35	52 B	54 B	72 C	72 A
	40	68 A	58 A	81 A	57 C
CV(%)		5,03			

Mean values followed by same tiny letter in the column and capital letter in the line does not differ at 5% level of probability.

CONCLUSION

The pre imbibition of oil radish seeds in water per 6 hours, the longitudinal cut in the higher direction and the 0,3% concentration of tetrazolium solution, in the temperature of 30°C can be used like methodology to evaluate the viability of oil radish lots, been this conditions recommended to be used in quality programs also.

RESUMO: A utilização do teste de tetrazólio é importante na avaliação da qualidade de lotes de sementes e vem sendo adotado para várias espécies na identificação do vigor e viabilidade. A adequação da metodologia do teste de tetrazólio para sementes de nabo forrageiro, espécie que vem se destacando como fonte de óleo para produção de biocombustíveis, poderia melhorar o processo de controle de qualidade. Além disso, a utilização do teste poderá fornecer subsídios para identificação de sementes remanescentes (mortas e dormentes) nos testes de germinação**.** Para verificar as condições ideais para a realização do teste de tetrazólio, em um 1º experimento as sementes de nabo forrageiro da cultivar CATI AL-1000, lotes de 2001 e 2006, foram submetidas à embebição entre papel em água por 6 horas. Após corte longitudinal no maior sentido as sementes foram imersas nas concentrações de 0,075%; 0,5% e 1,0% de solução de tetrazólio a 25ºC por 3 h, 12 h e 18 horas. Em um 2º experimento sementes de nabo forrageiro da cultivar CATI AL-1000, lote de 2005 e cultivar IPR 116 lotes de 2004 e 2005, foram imersas nas concentrações de 0,1%; 0,2%; 0,3% e 0,4% de solução de tetrazólio a 25°C, 30°C, 35°C e 40°C por 12 horas. Pelo 1º experimento foram observados a necessidade de testar concentrações intermediárias entre 0,075% e 0,5%, visto que, com 0,075% as sementes coloriram fracamente e com 0,5% os resultados do teste foram superestimados. No 2º experimento observou-se que a concentração 0,3% a 30°C pode ser recomendada para utilização no teste de tetrazólio para avaliação da viabilidade de sementes de nabo forrageiro.

PALAVRAS-CHAVE: *Raphanus sativus*. Pré-condicionamento. Semente.

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