ASSESS THE DROUGHT TOLERANCE OF BC15 RICE VARIETIES USING INVITRO CULTURE METHOD

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SUMMARY

BC15 rice is a pure quality rice variety, capable of adapting to large ecological ranges, therefore chosen by farmers to cultivate on large areas. Because global warming and climate change have greatly affected the water source for irrigation, so the rice yield is significantly reduced. This study was conducted to evaluate the drought tolerance of the BC15 variety in vitro culture system to get more information about the characteristics of the BC15 variety when selecting production areas. The object of the study was to determine the most suitable artificial drought conditions for BC15 rice seedlings in vitro. The result shows that in the MS1/2 medium supplemented with PEG with osmotic potential -4 bar; or the MS1/2 supplemented with 7% Saccharose; or the MS1/2 added 3% Mannitol or MS1/2 added 4% Sorbitol, shoots of the rice can be survival. Under these artificial drought conditions caused by Saccharose, Mannitol, Sorbitol, Mannitol expressed the strongest effect on rice sprouts in vitro. Just adding 3% Mannitol to the medium, the root length and shoot height were significantly reduced compared with the control, followed by Sorbitol and finally Saccharose, significantly on the roots and shoots of the seedlings. The results of this study will support necessary information about the suitable environment to evaluate the drought tolerance of rice in vitro and the basic source for in vivo assessment.

Keywords: BC15, drought tolerance, *in vitro, Oryza Sativa L.*, rice.

1. INTRODUCTION

Rice (*Oryza Sativa* L.) is the main food source for more than half of the world's population. In Vietnam, rice is an agricultural crop that plays an important role in the national economy. Rice farming in Vietnam dates back to ancient times and is a center of diversity in rice cultivation today. With an annual planting area of about 7.4 million hectares, mainly concentrated in the two regions of the Mekong River Delta (3.8 million hectares) and the Red River Delta (1.2 million hectares) (Nguyen Van Luat, 2009).

BC15 is a pure rice variety selected and bred by the Center for Agriculture and Fisheries Extension of Thai Binh province, selected and domesticated by Thai Binh Seed Joint Stock Company; officially recognized by the Ministry of Agriculture and Rural Development since December 2008. This rice variety was discovered and selected from a natural mutation of IR17494. BC15 is a short-term rice variety (equivalent to rice variety Q5), with stable purity, high yield, strong tillering, fairly resistance to leaf blight, dry blight, resistance to planthoppers, wide adaptability, especially rice in and thick sticky rice.

With the current situation of water scarcity in agricultural cultivation. Simultaneously with the complicated terrain conditions in our country, three-quarters of the territory is mountainous, the terrain is divided and the climate is complicated, along with climate change, the rainfall is unevenly distributed. Therefore, drought can occur at any time, in any region of the year, so it is necessary to select and breed drought-resistant rice varieties. At present, BC15 variety has become the mainstay variety of many localities across the country, so assessing the drought tolerance of BC15 variety is necessary, especially during the very sensitive period of rice plants to drought, which is germination and drought. seedling. Therefore, we performed this study to evaluate the drought tolerance of BC15 in an in vitro culture system to understand the growth of rice sprouts under artificial drought conditions and the choice of a suitable medium for research on drought tolerance of rice.

2. RESEARCH METHODOLOGY

2.1. Material

BC15 rice variety supported by Thai Binh Seed Corporation.

2.2. Research Methods

2.2.1. the basis culture mediums

The suitable culture medium for *in vitro* growth of rice sprouts is MS1/2 medium (with micromineral contents reduced by 1/2) + 30g/l Saccharose + 8g/l Agar (Nguyen Thi Luong,

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2013; Hoang Thi Ngoc Phuc, 2013). The culture mediums were adjusted to pH 5.8, autoclaved at 121^oC, 1 atm, then poured into bags. Culture conditions were 25 ± 2^0C, with 2000 ± 200 lux.

2.2.2. Methods of sample preparation and sterilization

Sample preparation The rice grains were dehulled, then the seeds were selected and the embryos were still intact, free from fungal and bacterial diseases. They were disinfected with 10% sodium hypochlorite (NaClO) in a sterile incubator.

2.2.3. Experimental methods

Evaluation of the growth ability of BC15 rice sprouts in the MS1/2 medium supplemented with PEG with different concentrations.

Rice seeds inoculated on MS ½ medium supplemented with 30g/l Saccharose and 8g/l Agar added PEG with different concentrations which created different osmotic potentials such as 0 (distilled water), -2, -4, -6 bar, corresponding to PEG content as 0 g/l; 119.6 g/l; 178.4 g/l and 223.7 g/l PEG, respectively. Each recipe was a culture of 15 seeds, repeated 3 times.

The osmotic potential by PEG-6000 was calculated according to the following formula (Burlyn E et al., 1973).

Ψ_s = -(1.18 x 10^{-2}) C - (1.18 x 10^{-4})C^2 + (2.67 x 10^{-4}) CT + (8.39 x 10^{-7}) C^2T

In which, C is the concentration of PEG-6000 in 1 little of H_2O; T is the temperature of the germination medium.

Evaluation of the growth ability of BC15 rice sprouts in the mediums supplemented with Saccharose, Mannitol, Sorbitol with different concentrations.

Rice seeds inoculated on the MS1/2 medium added 8g/l agar with different saccharose concentrations of 0%, 1%; 2%; 3%; 4%; 5%; 6%; 7%, or mannitol with concentrations of 0%; 1%; 2%; 3%; 4% and sorbitol with concentrations of 0%; 1%; 2%; 3%; 4%. Each recipe was a culture of 15 seeds, repeated 3 times.

2.2.4. Analyze and process data

a) Root lenght (average) =

$\sum root\ length / \sum number\ of\ root$ (cm).

It was measured root-to-root tip of experimental plants.

b) Shoot length (average) =

$\sum shoot\ length / \sum number\ of\ shoot$ (cm).

It was measured from base to shoot tip of experimental plants.

c) Germination rate =

$\sum number\ of\ gerninated\ seed / \sum number\ of\ cultured\ seed$ (%).

It was calculated when the experimental plants have sprouts and roots about 1mm long.

The data were processed and analyzed by Microsoft Excel 2010 and IRRISTAT 5.0 statistical software.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the growth of BC15 rice sprouts in medium supplemented with PEG with different concentrations

After culturing rice variety BC15 on the MS1/2 media supplemented with different PEG. The results are presented in Table 1.

Table 1. Growth of BC15 rice sprouts after 7 days of culture in MS1/2 medium with different concentrations of PEG

Formular	PEG-6000 (g/l)	Osmotic potential (bar)	Germination rate (%)	Root length (cm)	Shoot height (cm)
CT1	0	0	100	3.65 ±0.07 ^{a}	7.70±0.20 ^{a}
CT2	119.6	-2	93.33	3.00 ±0.04 ^{ab}	7.09±0.02 ^{b}
CT3	178.4	-4	66.67	2.64 ±0.02 ^{b}	4.50±0.09 ^{c}
CT4	223.7	-6	46.67	1.86 ±0.03 ^{b}	3.23±0.07 ^{d}
LSD 0.05				0.9	0.25
CV (%)				1.6	2.2

MS1/2 environment (Murashige and Skoog, 1962)

The letters a, b, c… in the same column represent a significant difference with α = 0.05.

The figure from Table 1 shows that the smaller the osmotic potential was, the harder it was for the seeds to get water from the environment, thus, the germination rate of seeds also decreased. Germination rate decreased from 100% in the control formula (0 bar) to 93.33% (-2 bar); 66.67% (-4 bar) and less than 50% when the osmotic potential was -6 bar.

After 7 days of culture, the root length and shoot height were different between the experimental treatments. At the osmolarity of -2 bar (119.6 g/l PEG) there was no difference with the control formula. The root length and shoot height of BC15 seedlings decreased gradually at -4 bar and the lowest at -6 bar (the average root length was only 1.86 cm and the average shoot height was 3.23 cm. This result shows that BC15 has good drought tolerance, consistent with the study of Vu Ngoc Thang et al. (2011) on mung beans at -6 bar, the germination rate of green beans decreased sharply, the root length was less than 3cm and the sprout length was less than 0.5 cm.

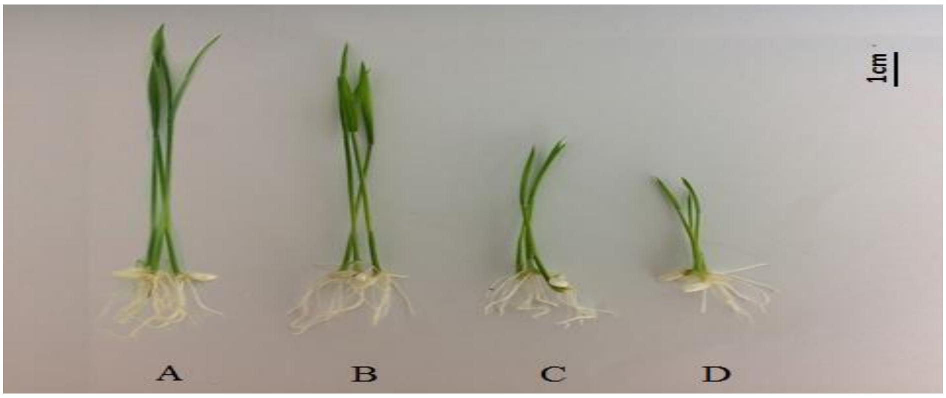

Figure 1. BC15 rice sprouts *in vitro* after 7 days of culture on MS1/2 medium supplemented with PEG with different concentrations

(A, B, C, D- rice sprouts at osmotic potentials 0, -2, -4, -6 (bar), respectively)

3.2. Evaluation of growth of BC15 rice sprouts in the medium supplemented with Saccharose, mannitol, and sorbitol with different concentrations

Evaluation of growth of BC15 seedlings on the MS1/2 mediums supplemented with Saccharose, Mannitol and Sorbitol with different concentrations, the results are presented in Table 2.

Table 2 demonstrates that after 3 days of culture, there was a slight difference between the mediums with different concentrations of Saccharose in which the root length and the shoots of rice sprouts grew strongly, quickly. Root length parameter was different among the mediums supplemented with different concentrations of saccharose (2% to 7%) compared with that of the control and the mediums supplemented with 0%, 1% Saccharose. The root length increased when adding over 2% Saccharose, and the highest in the medium added 3% Saccharose, reached 3.76±0.02 cm. Similarly, shoot height in the medium with 3 or 4% Saccharose was highest, around 3.8 cm. It decreased gradually as the concentration of Saccharose was over 5%, and especially most pronounced in the medium with 7% Saccharose, with 0.78 cm only.

After 7 days of culture, the shoot height changed significantly. There was a difference in root length parameters in the mediums. When adding 2% and 3% Saccharose to the medium, there was the highest point in shoots' root length, about 4.8 cm. When the concentration of Saccharose increased from 4% to 7%, the root length decreased gradually. In the medium of 7% Saccharose, the decrease in root length was most pronounced. The shoot height of shoots was also not the same in the mediums. In the mediums with concentrations of 1%, 2%, or 3%

of Saccharose, there was no obvious difference, and all made the highest, over 9 cm. When the concentration of Saccharose increased above 4%, the shoot height decreased gradually, and in the medium with 7% Saccharose, the shoot height decreased significantly, 3.19 cm only.

Table 2. Growth of BC15 rice sprouts after 3.7 days of culture in MS1/2 medium with different concentrations of Saccharose, Mannitol and Sorbitol

Formular	Chemical	Concentration (%)	Germination rate (%)	Root Length(cm)		Bud length(cm)	
				After 3 days	After 7 days	After 3 days	After 7 days
CT1	Saccharose (I)	0	100	1.36±0.05 ^{fI}	3.65±0.07 ^{dD}	0.98±0.02 ^{bcC}	7.70±0.20 ^{cC}
CT2		1	100	1.36±0.04 ^{fI}	4.01±0.07 ^{cC}	1.21±0.07 ^{bCBC}	9.11±0.06 ^{aA}
CT3		2	100	1.95±0.05 ^{dF}	4.84±0.02 ^{aA}	1.63±0.02 ^{bCBC}	9.11±0.09 ^{aA}
CT4		3	100	3.76±0.02 ^{aA}	4.88±0.06 ^{aA}	3.80±0.07 ^{aA}	9.22±0.03 ^{aA}
CT5		4	100	3.59±0.02 ^{bB}	4.46±0.03 ^{bB}	3.74±0.09 ^{aA}	8.47±0.01 ^{bB}
CT6		5	100	2.76±0.06 ^{cC}	4.03±0.10 ^{cC}	1.83±0.03 ^{bB}	7.06±0.02 ^{dD}
CT7		6	93.33	2.60±0.01 ^{cD}	4.02±0.02 ^{cC}	1.79±0.03 ^{bB}	6.24±0.15 ^{eH}
CT8		7	73.33	1.73±0.22 ^{eG}	3.19±0.03 ^{eE}	0.78±0.08 ^{cC}	3.96±0.06 ^{fK}
	LSD 0.05 (I)			0.16	0.11	0.97	0.18
	CV % (I)			3.8	1.5	2.8	1.3
CT9	Mannitol (II)	0	100	1.36±0.04 ^{abI}	3.65±0.05 ^{aD}	0.98±0.01 ^{bC}	7.70±0.03 ^{aC}
CT10		1	100	1.78±0.05 ^{aG}	2.98±0.13 ^{bF}	1.40±0.01 ^{aBC}	7.62±0.14 ^{aC}
CT11		2	93.33	1.81±0.04 ^{aG}	2.81±0.12 ^{bF}	0.91±0.04 ^{bC}	6.51±0.05 ^{bF}
CT12		3	66.67	1.56±0.07 ^{aH}	2.40±0.14 ^{cG}	0.86±0.02 ^{bC}	3.59±0.18 ^{cM}
CT13		4	44.44	0.75±0.03 ^{bK}	1.36±0.07 ^{eI}	0.56±0.02 ^{cC}	1.53±0.07 ^{dN}
	LSD 0.05 (II)			0.8	0.18	0.25	0.21
	CV% (II)			2.9	3.7	1.4	2.1
CT14	Sorbitol (III)	0	100	1.36±0.02 ^{bI}	3.65±0.04 ^{aD}	0.98±0.08 ^{cC}	7.70±0.01 ^{aC}
CT15		1	100	2.24±0.07 ^{aE}	3.20±0.05 ^{bE}	2.17±0.03 ^{aB}	6.83±0.15 ^{bE}
CT16		2	93.33	1.99±0.03 ^{abF}	2.40±0.04 ^{cG}	1.61±0.02 ^{bBC}	6.36±0.12 ^{cG}
CT17		3	66.67	1.53±0.01 ^{abH}	2.35±0.06 ^{cG}	1.10±0.05 ^{bcC}	5.62±0.02 ^{dI}
CT18		4	60	1.36±0.01 ^{bI}	1.71±0.02 ^{dH}	0.88±0.01 ^{cC}	3.77±0.07 ^{eL}
	LSD 0.05 (III)			0.79	0.11	0.53	0.26
	CV % (III)			2.5	2.1	2.1	2.3
	LSD 0.05			**0.11**	**0.2**	**0.68**	**0.12**
	CV %			**3.5**	**1.8**	**2.7**	**2.2**

The letters a, b, c… in the same column represent the significant difference of each group of formula 1 (CT1, CT2…CT8), group of formula II (CT9, CT10…CT13), group of formula III (CT14, CT5... CT18) with α = 0.05.

The letters A, B, C... in the same column represent the significant difference of the same chemical group with α = 0.05.

In short, on the MS1/2 medium supplemented with 3% Saccharose, seedling developed best, and MS1/2 medium supplemented with 7% Saccharose, seedling developed slowest. This result was similar to the study of Nguyen Thi Luong (2013) who announced that in the MS1/2 medium supplemented with 3% Saccharose, Oryza

Sativa L sprouts grew best after 7 days, the root length was 7.36±0.02 cm and shoot height was 14.67±0.03 cm.

The growth of BC15 rice sprouts in the medium supplemented with Mannitol with different concentrations is shown in Table 2. It is shown that, after 3 days of culture, the root length and shoot height grew fast as well. The root length did not have much difference compared with the control when adding Mannitol concentration from 1% to 3%. Only when increasing the concentration of Mannitol to 4%, this difference was significant. When the concentration of mannitol increased above 2%, the root length started to decrease, meanwhile, the shoot height was different between MS1/2 medium supplemented with 1% Mannitol compared to the control, but as concentration increased over 2%, shoot height decreased.

After 7 days of culture, root growth slowed down, shoot height grew fast, and became strong. Root length was different between the shoots in MS1/2 medium supplemented with Mannitol and compared with the control, no Mannitol at al. When increasing the concentration of Mannitol, the root length also decreased. Meanwhile, it was significant to add more than 2% of Mannitol for shoots to have a different growth. The higher the mannitol concentration was, the shorter the shoot height was.

The medium supplemented with Mannitol 3% showed the most observable effects. This result was completely consistent with the research results of Nguyen Thi Luong (2013).

Table 2 also illustrates the growth of BC15 rice sprouts in the medium supplemented with Sorbitol with different concentrations. It can be seen that after 3 days of culture, the root length and the shoot height were also affected by the different concentrations of Sorbitol. Specifically, the root length of BC15 rice sprouts in MS1/2 medium supplemented with Sorbitol at 1 to 3% (around 2 cm) was significantly different from that of the control, 1.36 cm. However, in the medium with 4 mg/l Sorbitol, the root length was 1.36 cm also. In terms of shoot height, the medium of 1 mg/l Sorbitol made up the highest, 2.17 cm and the lowest referred to the shoots cultured in the medium with 4 mg/l Sorbitol.

After 7 days of culturing, it was evident that the medium with 1 to 4% Sorbitol inhibited the root length and shoot height. The higher the sorbitol concentration was, the shorter the root length and shoot height were. The medium supplemented with Sorbitol 4% showed the most negative effect, the shoot height and root length were a half compared to the figure of the control.

In general, all their mediums supplemented with Saccharose, Mannitol, Sorbitol affected the growth of BC15 rice shoots.

After 3 days of culturing, in the mediums of 3% Saccharose, the root length and shoot height of BC15 seedlings was the highest, 3.76 cm, and 3.8 cm, respectively. However, the lengthening of roots was inhibited in the medium with Mannitol and Sorbitol. In the mediums with 4% Mannitol or 4% Sorbitol, the root length was 0.75 cm and 1.36 cm, respectively, and the shoot height of shoots in the former was 0.56 cm and in the latter was 0.88 cm.

After 7 days of culturing BC15 rice seeds, the data shows the obvious effects of three kinds of sugar which made artificial drought conditions on BC15 shoots in vitro. Especially, in the medium supplemented with 7% Saccharose, 4% Mannitol, or 4% Sorbitol, rice sprouts grew badly. In the medium supplemented with 7% Saccharose, although root length was a little smaller than that of the control, seedling rice grew as half as the control as shoot height of them was 3.96±0.06 cm, while the control was 7.7±0.2 cm. Then, the medium supplemented with 4% Sorbitol express the inhibition to root length and shoot height with 1.71±0.02 cm, and 3.77±0.07 cm, respectively which were around as half as the control, the figure of root and shoot were 3.65±0.04 cm and 7.7±0.01 cm. Finally, in the medium supplemented with 4% Mannitol, root length was reported was 1.36±0.07 cm and shoot height was 1.53±0.07 cm, which was evident of the strong inhibition of Mannitol on BC15 seedling.

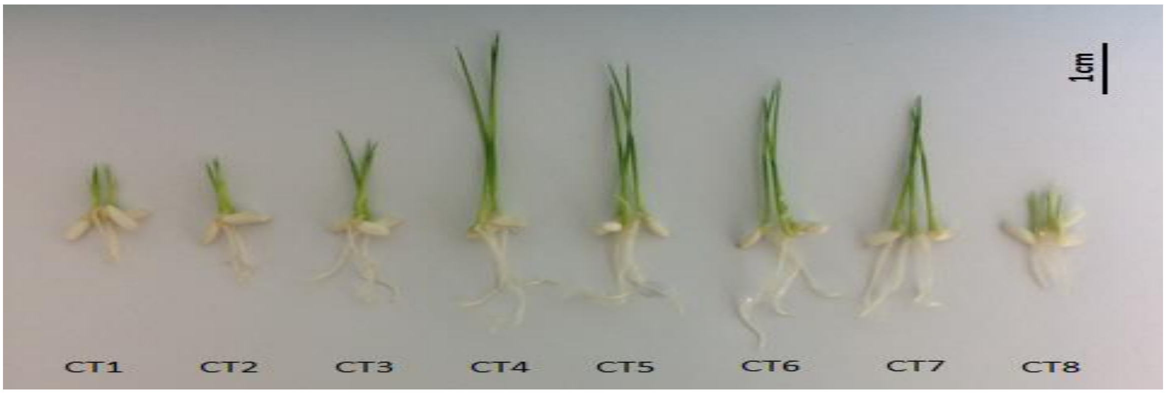

Figure 2. BC15 rice sprouts *in vitro* after 3 days of culture on MS1/2 medium supplemented with different concentrations of Saccharose

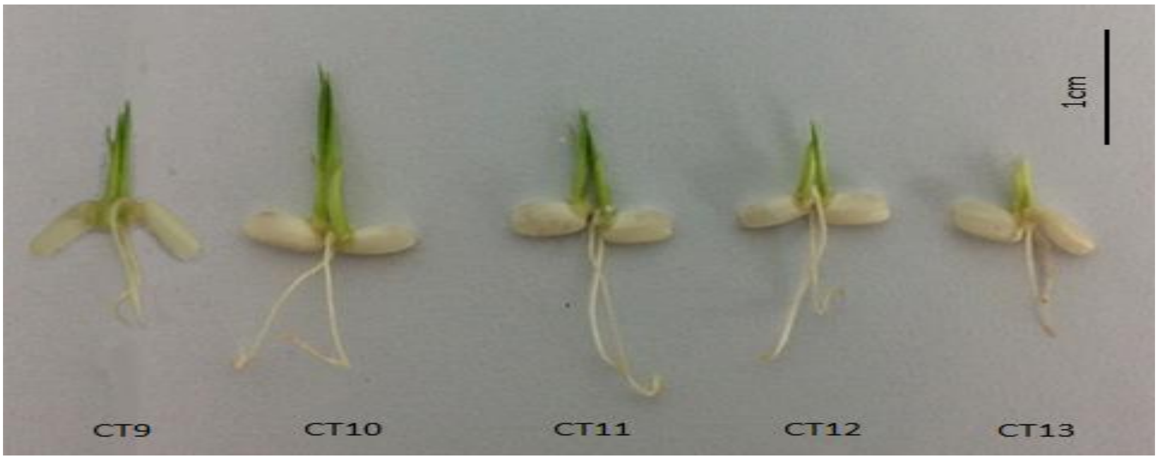

Figure 3. BC15 rice sprouts *in vitro* after 3 days of culture on MS1/2 medium supplemented with mannitol with different concentrations

Figure 4. *In vitro* BC15 rice sprouts after 3 days of culture on MS1/2 medium supplemented with Sorbitol with different concentrations

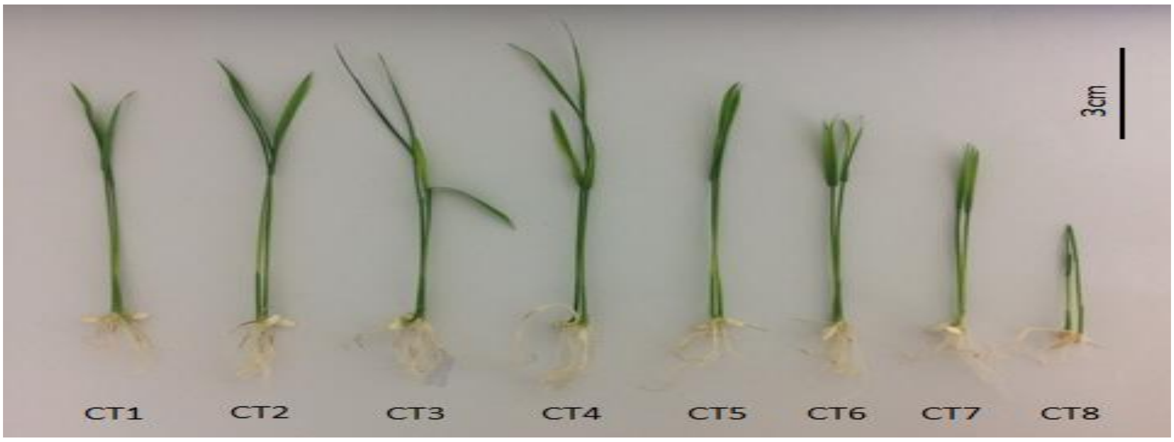

Figure 5. **BC15 rice sprouts *in vitro* after 7 days of culture on MS1/2 medium supplemented with different concentrations of Saccharose**

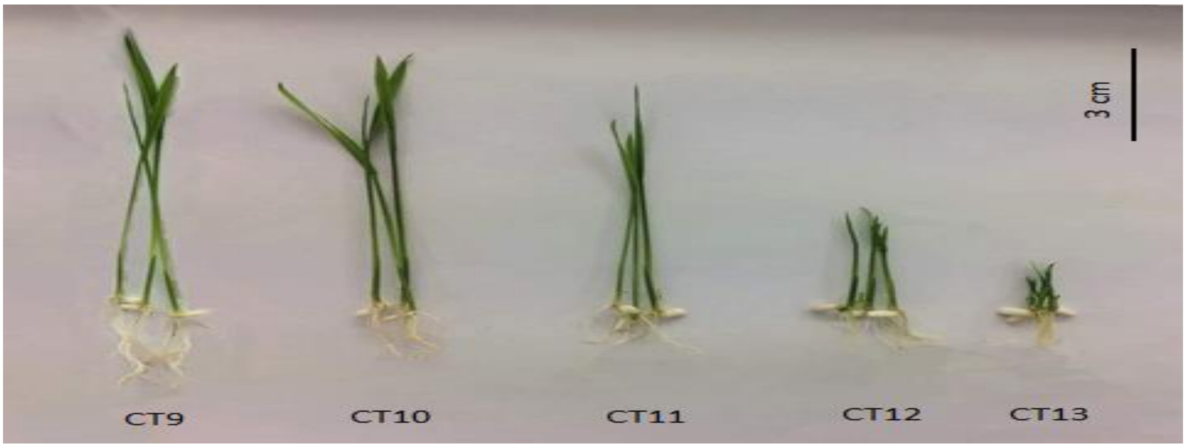

Figure 6. **BC15 rice sprouts *in vitro* after 7 days of culture on MS1/2 medium supplemented with mannitol with different concentrations**

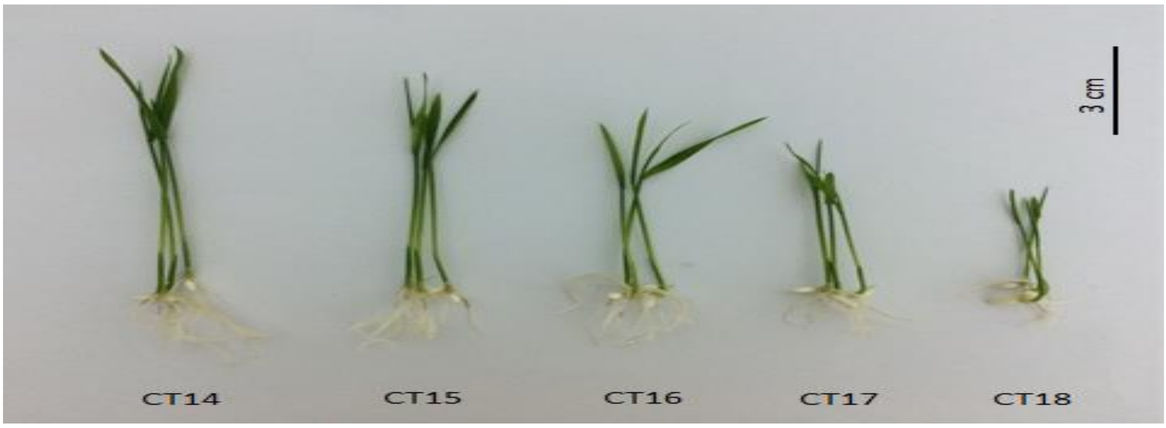

Figure 7. **BC15 rice sprouts *in vitro* after 7 days of culture on MS1/2 medium supplemented with Sorbitol with different concentrations**

4. CONCLUDE

Studying the drought tolerance of BC15 rice variety in vitro, there are some following conclusions:

1. That the artificial drought conditions can be used for examination of drought tolerance of BS15 rice variety was the MS1/2 supplemented with PEG. The higher the PEG concentration was, the lower the growth of rice sprouts was. At the osmotic potential -4 bar, the decrease in root length and shoot height was the most observed, shoot height was 4.5±0.09 cm and root length was 2.64 ±0.02 cm

2. Under artificial drought conditions, the

MS1/2 supplemented with Saccharose, rice sprouts grow best when the concentration of Saccharose was 3% in which the root length was 4.88±0.06 cm, and shoot height was 9.22±0.03 cm. After 7 days, the medium with Saccharose of 7%, the decrease in shoot height and root length was most pronounced, with root length of 3.19±0.03 cm and shoot height of 3.96±0.06 cm.

3. MS1/2 supplemented with Mannitol made artificial drought conditions for the growth of the rice sprouts in vitro. Mannitol 3% caused the most obvious drought expression, with the shoot being 3.59±0.18 cm in height and the root of 2.4±0.14 cm in length after 7 days of culturing.

4. The higher the concentration of Sorbitol in the MS1/2 affected the growth of rice sprouts was as well. 4% Sorbitol in the culturing medium inhibited the growth of seeding rice with 3.77±0.07 cm shoot height and 1.71±0.02 cm root length.

5. All Saccharose, Mannitol, and Sorbitol can be artificial drought conditions to examine rice sprouts which made them all grow and develop poorly. Mannitol sugar caused the strongest effect on rice sprouts, just adding 3% Mannitol to the medium, the root length and shoot height decreased sharply, followed by 4% Sorbitol and finally 7% Saccharose.

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ĐÁNH GIÁ KHẢ NĂNG CHỊU HẠN IN VITRO CỦA GIỐNG LÚA BC15

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TÓM TẮT

Giống lúa BC15 là giống lúa thuần chất lượng, có khả năng thích nghi với nhiều vùng sinh thái và được người dân lựa chọn canh tác với diện tích lớn. Do thực trạng trái đất nóng lên, khí hậu bị biến đổi ảnh hưởng lớn đến nguồn nước tưới nên năng suất lúa bị giảm đáng kể. Nghiên cứu này được thực hiện nhằm đánh giá khả năng chịu hạn của giống BC15 trong hệ thống nuôi cấy invitro để có thêm thông tin về đặc điểm của giống BC15 khi lựa chọn vùng sản xuất. Kết quả nghiên cứu là đã xác định được các điều kiện hạn nhân tạo thích hợp cho cây mầm lúa BC15 trong điều kiện nuôi cấy invitro, đó là môi trường MS1/2 bổ sung PEG với thế thẩm thấu -4 bar, MS1/2 bổ sung 7% Saccharose, MS1/2 bổ sung 3% Mannitol và MS1/2 bổ sung 4% Sorbitol. Trong điều kiện hạn nhân tạo gây ra bởi Saccharose, Mannitol, Sorbitol, thì đường Mannitol gây ra tác động mạnh nhất trên cây mầm lúa. Chỉ cần bổ sung Mannitol 3% vào môi trường thì chiều dài rễ và chiều dài chồi đã giảm mạnh so với đối chứng, tiếp đến là Sorbitol và cuối cùng là Saccharose, phải bổ sung Saccharose 7% vào môi trường thì mới gây tác động rõ rệt lên rễ và chồi của cây mầm so với đối chứng. Kết quả nghiên cứu này sẽ bổ sung thông tin về môi trường để đánh giá khả năng chịu hạn của cây lúa bằng nuôi cấy *in vitro* và phục vụ đánh giá ngoài đồng ruộng.

Từ khóa: BC15, chịu hạn, *in vitro*, lúa, *Oryza Sativa L.*

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