

IMPROVING SEEDLING GROWTH AND TILLERING WITH OSMOTIC PRIMING TREATMENTS IN RICE CV. HAMZADERE AND OSMANCIK 97

M. P. Kahriz and P. P. Kahriz*

Department of Field Crops, Faculty of Agriculture, Ankara University, 06110, Dışkapı, Ankara, Turkey
Corresponding author's email: mahsapourali7@gmail.com

ABSTRACT

Rice (*Oryza sativa* L.) is mainly cultivated in Thrace, Black sea region and many land locked small pockets throughout Turkey. Cultivar Hamzadere and Osmancik 97 are important rice cultivars that are resistant against lodging and are moderately tolerant to blight and root rot along with high yield potential. To synchronise uniform stand, planting of rice seedlings are preferred over direct seeding. Rice is very sensitive to water shortage. Good stand results in good crop. The study aimed to identify a system that could induce high tillering and accelerated growth of plants during early stages of growth at seedling stage. The seeds of two cultivars were primed on semi solid agar gelled MS medium or MS medium having dissimilar doses of N⁶-Benzyladenine (BA) and Naphthaleneacetic acid (NAA) used singly or in combination to improve earlier seedling growth and tillering. The results of the study showed variable effects of priming on growth components and parameters depending on concentration of plant growth regulators used. BA+NAA priming was inhibitory to the growth and development of seedlings' shoots and roots of both cultivars. BA treatments used singly were comparatively better; however, the treatments that contained NAA used singly in any concentration or MS medium used singly had significant edge over rest of the treatments. A significant and distinct improvement was observed in terms of all growth parameters including number of tillers per seedling. It is concluded that priming on nutrient rich MS medium could help in uniformity of growth and seedling development without any bottleneck and improved yield.

Key words agar gelled priming, auxins, cytokinins, multiplication, stress, tillers, fresh weight, dry weight

Abbreviations BA: 6 benzyladenine, NAA: 1 Naphthaleneacetic acid.

INTRODUCTION

There are 20 species in genus *Oryza* with a basic chromosome number of 12 (Watanabe *et al.*, 1994; 1997). They grow in areas with evenly distributed rainfall. Rice is one of the world's most important food grain cereal that is consumed by more than a third population. Most (90%) of rice is produced and consumed in Asian countries. Only a very small amount (about 5%) of rice is internationally exported but its production and prices remain unstable (FAO, 2016). All rice cultivars grown in Turkey are of Japonica/Sinica origin, with moderate tillering. They grow well in low temperatures regions and have short round grains with low amylose content (Small, 2009).

There is need to develop cost effective, long run integrated biotechnological and traditional breeding technologies that could help in improved, rapid and uniform growth of rice with maximum water use efficiency, minimum weeds and increased productivity with little investment. Fast climatic variations due to greenhouse effect and scarcity of water with global food security are major concerns for rice growers worldwide.

Rice plant growth needs care at each stage of growth, that should be managed appropriately as rice is severely sensitive to water shortage. Thus water management is a critical aspect of rice farming as rice

plants are also able to fix free nitrogen from non biologic and biologic sources (Ladha and Reddy 2003).

Direct seedling growth is affected by erratic plant growth and poor seed germination along with use of high seed rate. As such direct seeded crops are not an attractive approach and the sown rice crop shows undesirable performance in fields (Farooq *et al.*, 2011). Moreover, seed priming increases seed vigor, allows selection of uniformly germinated seeds along with uniform planting and growth in the fields (Harris *et al.*, 2000; Harris *et al.*, 2001a; Harris *et al.*, 2001b). Harris and Mottram (2005) tested the germination response of water soaked rice seeds in an incubator at 30°C under *in vitro* conditions. They found that 8 hours priming was the most appropriate and 8-12 hours priming had significantly positive effects on seed germination. They also found that rice can be primed for upto 18 hours without significant deleterious effects on germination.

It is very difficult to perform field experiments and reach to a conclusive result due to variability in physical and chemical conditions of field soils, the physical and chemical properties that change variably at different temperatures. Therefore, there is every possibility of reaching a biased result in the field based experiments (Rengasamy 2002; Benderradji *et al.* 2012). It is desirable and easy to check the effects of culture conditions, nutrient and stress levels by carrying out

experiments under *in vitro* conditions to forestall these unaccounted biochemical and physiological responses in plants (Ahmad *et al.* 2007, Pe' rez-clemente and Go'mez-cadenas 2012). In line with these findings the study aimed to improve tillering and accelerated growth of cv. Hamzadere and Osmancik 97 by modified priming method using agar gelled MS medium, or MS medium having different concentrations of BA and NAA used singly or in combination.

MATERIALS AND METHODS

Plant Materials: The rice cultivars Hamzadere and Osmancik 97 used in the study were obtained from the Thrace Agricultural Research Institute (Trakya Tarımsal Araştırma Enstitüsü) Edirne, Turkey.

Cultivar Hamzadere was developed from a cross of line Demir × 83013-TR631-4-1-2 and registered by the Thrace Agricultural Research Institute during 2011. The plants of this cultivar are 95 cm tall, semi vertical and green with sturdy shoots. They have resistance against lodging. Rice grains are light yellow and long. Cultivar has 1000 grain weight of 37-38 gram and matures in 130 days with high yield potential. Cv. Hamzadere is tolerant to moderate blight and is resistant to root rot diseases. The rice grains are 6.6 mm in length with 2.9 mm stem width. Grain appearance is glossy and dull. (Anonymous, 2016a).

Cv. Osmancik 97 was developed by cross between cv. Rocca × Europa at the Agricultural Research Institute Thrace during 1997. It is 95-100 cm tall, and resistant to lodging with thousand grain weight of 33-34 g. It is medium early with ripening period of 130 -135 days. Rice grain is glossy in appearance. It yields 800-1000 kg per hectare or over, is moderately tolerant to stem rot and resistant to bunch blight. It is not affected by cold weather or cold watering conditions and contributes to over 75% rice production in Turkey. It is recommended for cultivation in almost all areas of Turkey, where rice farming is practised (Anonymous 2016b)

Priming of rice seeds Culture medium and culture conditions: The seeds of cv. Hamzadere were hulled before sterilization to avoid contamination with microorganisms like bacteria, fungus etc. followed by dipping them in 95% ethanol for 2 min and sterilized in 50% commercial bleach (5% NaOCl) for 15 min. Thereafter, the seeds were rinsed 3 ×3 min in bidistilled water. The seeds were cultured on MS medium (Murashige and Skoog 1962) in horizontal adaxial position having 1 mg/L BA (Control 1) or 1 mg/L BA + 0.01, 0.10, 0.15 mg/L NAA, 2 mg/L BA (Control 2) or 2 mg/L BA + 0.01, 0.10, 0.15 mg/L NAA, 3 mg/L BA (Control 3) or 3 mg/L BA + 0.01, 0.10, 0.15 mg/L NAA, 4 mg/L BA (Control 4) or 4 mg/L BA + 0.01, 0.10, 0.15

mg/L NAA, and MS medium (control 5), 0.01 mg/L NAA (Control 6), 0.10 mg/L NAA (Control 7) and 0.15 mg/L NAA (Control 8) and cultured under white fluorescent light giving 16 hours light photoperiod for 14 days. Besides determining effects of various growth parameters, fresh and dry weight of germinated seedlings was also evaluated.

The percentage of seed germination is an indicator of seed vigor; which was computed after five days (Anonymous, 2016c after modifications). Rest of the parameters described below were computed after 14 days of culture.

1. Germination percentage (computed after 5 days of culture)
2. Number of tillers per seed
3. Number of leaves per plant
4. Leaf length (cm)
5. Rooting percentage
6. Number of roots per plant
7. Root length (cm)
8. Fresh and dry weight of roots (gm)

All concentrations of BA, NAA were prepared from a 1 mg/ml stock solution prepared as per instructions of the manufacturers. All plant growth regulators were added before autoclaving. pH of the medium was adjusted to 5.7 ±0.1 using 1N NaOH or 1N HCl. All cultures were autoclaved at 104 kPa, 120°C for 20 min to sterilize them. Thereafter, they were poured in 10 × 100 mm glass Petri dishes in laminar flow cabins. The seeds were primed in horizontal adaxial orientation on culture plates. They were incubated under white fluorescent light using 16 h light photoperiod at 24±1°C.

Statistical analysis: Each treatment contained 100 seeds divided into 10 replicates (10 replications × 10 seeds =100 seeds) and were repeated 4 times. The experimental data were obtained after 14 days of culture. Statistical analysis was performed by taking means using One way Analysis of variance. The means were compared using Tukeys'b Test. All experimental values taken in percentage were subjected to arc sin square root transformation before subjecting them to statistical analysis following Snedecor and Cochran (1989).

RESULTS

Effects of different priming doses of BA, BA+NAA and NAA on Seeds of rice cv. Hamzadere: Although overall germination percentage of seeds varied between%33.26 - 93.33 (Table 1); yet the seeds germinated after priming with any dose of BA used singly (Control 1, 2, 3, 4) remained higher compared to seed germination realized on any cocentration of BA + NAA. All (100%) seeds germinated on MS medium (Control 5), MS medium having 0.01 mg/L NAA

(Control 6), MS medium having 0.10 mg/L NAA (control 7), and MS medium having 0.15 mg/L NAA (control 8).

Number of tillers per explant varied between 1.80 to 3.00 per seed germinated seedlings. No statistical difference was observed on shoots obtained on MS medium having 1 and 2 mg/L BA + varying concentrations of NAA. Again maximum number of tillers were realized on BA used singly (Control 1, 2, 3, 4) in respective groups, compared to when the BA was used with any concentration of NAA. It was observed that each rising concentration of NAA with any concentration of BA resulted in variable inhibition of germination percentage. Maximum number of tillers were sighted on MS medium having 3 mg/L BA and the minimum number of shoots were realized on MS medium having 4 mg/L BA+0.10 mg/L NAA. Whereas, 2.40, 2.80, 3.00 and 3.00 tiller shoots were observed on Control 1 or MS medium, Control 2, Control 3 and Control 4 respectively.

Shoot length extended between 2.73 - 7.23 cm. Again maximum shoot length in respective groups was realized on control 1, 2 and 3 treatments having BA used singly. Similarly, Maximum shoot length of 7.23, 7.14, 6.60 and 6.19 cm in all groups was sighted on Control 5 (MS medium), 6, 7 and 8 respectively that contained variable concentrations of NAA used singly.

Excluding non root inducing plants rooting percentage extended between 20 -100%. No rooting was realized on 1 and 2 mg/L BA + 0.10 ve 0.15 mg/L NAA, 3mg/L BA+0.15 mg/L NAA, and 4 mg/L BA + 0.10 or 0.15 mg/L NAA. 100% rooting was observed on 9 germination treatments. Rooting on BA used singly (Control 1, 2, 3, 4 treatments) and NAA used singly (Control 6, 7, and 8 treatments) showed 100% rooting. Rooting on Control 5 (MS medium) remained 80%. Priming with all concentrations of BA+NAA were either inhibitory or ended up with no roots.

Excluding non rooting plants, number of roots per explant extended between 0.200 - 12.40. Although low number of roots were seen on any culture that contained BA with any concentration of BA+NAA, yet the number of roots per explant were greater when BA was used singly in any concentration. Number of roots on Control 1 (MS medium), 2 (MS medium having 0.01 mg/L NAA), 3 (MS medium having 0.10 mg/L NAA) and 4 (MS medium having 0.15 mg/L NAA) remained 9.80, 11.20, 12.00 and 12.40 respectively. All of the roots were significantly longer compared to the seedlings developed on other treatments. These had pointed ends and were straight, strong and thick.

Root length extended between 0.06 - 11. 20 cm. Again longer roots of variable length were noticed when the BA was used singly (Control 1, 2, 3, and 4). Maximum root length was realized on Control 5, followed closely by control 6. Root length on control 7 and 8 showed sharp inhibition.

Fresh weight of seedlings remained 0.23 –0.98 g. Maximum fresh weight was observed on Control 5 (MS medium) followed closely by the fresh weight seen on control 6, 7 and 8 treatments. Although fresh weight sighted on BA used singly was sharply reduced but it remained higher to the fresh weight obtained on any concentration of BA+NAA.

Dry weight remained 0.08 - 0.16 g. Maximum dry weight was realized on 3 mg/L BA in MS medium and Control 5, 6 & 7. Although, sharp reduction in weight was observed on any concentration of BA or BA+NAA, again dry weight of seedlings obtained on control 1 (1 mg/L BA) and control 3 (3 mg/L BA) was higher in their respective groups.

Effects of different priming doses of BA, BA+NAA and NAA on Seeds of rice cv. Osmancik 97: The results showed germination percentage in range of 40.00 - 93.33%. Maximum germination was realized on MS medium having 2 mg/L BA + 0.15 mg/L NAA and 3 mg/L BA.

Number of tillers per seed varied between 2.20 to 3.20. Shoot length extended between 3.06 – 4.15 cm on MS medium having BA used singly or in combination with any concentration of NAA. In contrast, the seeds primed with MS medium (control 1) had shoot length of 9.26 cm, the seeds primed with MS medium having 0.01 mg/L NAA (control 2) had shoot length of 9.42 cm; the seeds primed with MS medium having 0.10 mg/L NAA (control 3) had shoot length of 5.91 cm and, the seeds primed with MS medium having 0.15 mg/L NAA (control 4) had shoot length of 6.13 cm.

Hundred (100%) rooting was noticed on plants induced after six treatments that included 4 control treatments 1, 2, 3 and 4 and MS medium having 1 and 4 mg/L BA used singly. No rooting was realized on 1 mg/L BA + 0.15 mg/L NAA, 4 mg/L BA + 0.15 mg/L NAA. Rooting percentage on plants induced on any concentration of BA or BA+NAA excluding control treatments 1, 2, 3 and 4 remained 20 to 100%

Excluding non-rooted plants and controls 1, 2, 3 and 4, the number of roots varied between 0.40 to 1.80 per seed induced plant. MS medium (control 1) treated seeds induced 5.80 roots per plant. Total number of roots on control 2, 3, and 4 were 10.00, 10.20 and 5.80 respectively.

Root length extended between 0.11 – 4.07 cm. The longest roots were observed on MS medium (control 1). Root length on control 2, 3 and 4 treatments remained 1.90, 0.68 and 0.93 cm respectively.

Fresh root weights varied between 0.22 to 0.39 g. Fresh weight on control 1, 2, 3 and 4 treatments was 0.53, 0.64, 0.60 and 0.53 g respectively.

Dry weight had range of 0.09 - 0.14 g. The maximum dry weight was realized on MS medium having 1 mg/L BA + 0.10 mg/L NAA.

Table 1. Effects of hormonal priming of seeds on semi solid agar solidified MS medium on cv. Hamzadere cultured in horizontal adaxial position.

BA (mg/L)	NAA (mg/L)	Seed germination percentage (%) [*]	Number of tillers per explant [*]	Shoot length (cm) ^{**}	rooting percentage (%) ^{**}	Number of roots per plant ^{**}	Root length (cm) ^{**}	Fresh weight (g) ^{**}	Dry weight (g) ^{**}
1.00 (Control 1)		86.52 ^{ab}	2.60 ^{ab}	4.51 ^{cdef}	100.00 ^a	2.60 ^c	2.60 ^{bc}	0.38 ^d	0.13 ^{bc}
1.00	0.01	73.39 ^{cd}	2.40 ^{ab}	2.91 ^f	100.00 ^a	1.60 ^c	0.97 ^{bcd}	0.27 ^j	0.11 ^{de}
1.00	0.10	66.50 ^{de}	2.40 ^{ab}	3.21 ^f	0.00 ^b	0.00 ^c	0.00 ^c	0.27 ^j	0.11 ^{de}
1.00	0.15	47.52 ^g	2.40 ^{ab}	3.09 ^f	0.00 ^b	0.00 ^c	0.06 ^c	0.29 ^{ij}	0.11 ^{de}
2.00 (Control 2)		86.66 ^{ab}	2.80 ^{ab}	4.69 ^{bcd}	100.00 ^a	1.60 ^c	2.99 ^b	0.37 ^{de}	0.10 ^{ef}
2.00	0.01	75.53 ^{bcd}	2.40 ^{ab}	2.73 ^f	100.00 ^a	1.40 ^c	2.44 ^{bcd}	0.32 ^{gh}	0.10 ^{ef}
2.00	0.10	66.51 ^{de}	2.20 ^{ab}	4.45 ^{cdef}	0.00 ^b	0.00 ^c	0.00 ^c	0.35 ^{ef}	0.11 ^{cd}
2.00	0.15	33.26 ^h	2.20 ^{ab}	3.43 ^{ef}	0.00 ^b	0.00 ^c	0.00 ^c	0.31 ^{hi}	0.14 ^b
3.00 (Control 3)		93.33 ^a	3.00 ^a	5.85 ^{abcd}	100.00 ^a	1.60 ^c	2.55 ^{bc}	0.39 ^d	0.16 ^a
3.00	0.01	82.00 ^{bc}	2.00 ^{ab}	5.50 ^{abcde}	80.00 ^a	1.20 ^c	0.78 ^{bcd}	0.34 ^{fg}	0.09 ^{fg}
3.00	0.10	80.00 ^{bc}	2.00 ^{ab}	3.51 ^{ef}	80.00 ^a	1.80 ^c	0.20 ^{de}	0.26 ^j	0.08 ^g
3.00	0.15	52.93 ^g	2.60 ^{ab}	4.85 ^{bcd}	0.00 ^b	0.00 ^c	0.00 ^c	0.32 ^{gh}	0.11 ^{de}
4.00 (Control 4)		93.39 ^a	2.40 ^{ab}	3.69 ^{ef}	100.00 ^a	1.00 ^c	1.00 ^{bcd}	0.32 ^{gh}	0.11 ^{de}
4.00	0.01	53.33 ^{fg}	2.00 ^{ab}	3.68 ^{ef}	20.00 ^b	0.20 ^c	0.26 ^{de}	0.23 ^k	0.10 ^{ef}
4.00	0.10	61.20 ^{ef}	1.80 ^b	4.07 ^{def}	0.00 ^b	0.00 ^c	0.00 ^c	0.27 ^j	0.11 ^{de}
4.00	0.15	53.26 ^{fg}	2.00 ^{ab}	4.80 ^{bcd}	0.00 ^b	0.00 ^c	0.00 ^c	0.31 ^{hi}	0.12 ^{cd}
Control 5-MS medium		100.00 ^a	2.40 ^{ab}	7.23 ^a	80.00 ^a	9.80 ^b	9.80 ^a	0.98 ^a	0.16 ^a
Control 6	0.01	100.00 ^a	2.80 ^{ab}	7.14 ^a	100.00 ^a	11.20 ^{ba}	11.20 ^a	0.80 ^b	0.16 ^a
Control 7	0.10	100.00 ^a	3.00 ^a	6.60 ^{ab}	100.00 ^a	12.00 ^{ba}	0.72 ^{bcd}	0.90 ^b	0.16 ^a
Control 8	0.15	100.00 ^a	3.00 ^a	6.19 ^{abc}	100.00 ^a	12.40 ^a	0.58 ^{cdc}	0.81 ^c	0.12 ^{cd}

**Values showed in same column by different letters are statistically different using Tukeys' b Test at 0.01 level of significance

*Values showed in same column by different letters are statistically different using Tukeys' b Test at 0.05 level of significance.

All values are means of 100 explants repeated 4 times, All means for fresh and dry weigh are means of 5 explants repeated 4 times

Control 1 = MS mdium having 1 mg/L BA, Control 2 = MS mdium having 2 mg/L BA, Control 3 = MS mdium having 3 mg/L BA,

Control 4 = MS mdium having 4 mg/L BA, Control 5 = MS mdium, Control 6 = MS mdium having 0.01 mg/L NAA,

Control 7 = MS mdium having 0.10 mg/L NAA, Control 8 = MS mdium having 0.15 mg/L NAA

Table 2 4.11. Effects of hormonal priming of seeds on semi solid agar solidified MS medium on cv. Osmancik 97 cultured in horizontal adaxial position.

BA (mg/L)	NAA (mg/L)	Seed germination percentage (%) [*]	Number of tillers per explant [*]	Shoot length (cm) ^{**}	rooting percentage (%) ^{**}	Number of roots per plant ^{**}	Root length, (cm) ^{**}	Fresh weight (g) ^{**}	Dry weight (g) ^{**}
1.00 (Control 1)		66.66 ^{de}	2.80 ^{ab}	3.73 ^c	100.00 ^a	1.60 ^c	2.57 ^b	0.26 ^{hij}	0.11 ^{cd}
1.00	0.01	46.66 ^{fj}	3.00 ^a	3.23 ^c	80.00 ^{ab}	1.40 ^c	0.60 ^{cd}	0.24 ^{ij}	0.11 ^{cd}
1.00	0.10	40.00 ^j	3.00 ^a	3.67 ^c	80.00 ^{ab}	1.60 ^c	0.22 ^d	0.31 ^{fj}	0.14 ^a
1.00	0.15	60.00 ^e	2.80 ^{ab}	3.26 ^c	0.00 ^b	0.00 ^c	0.00 ^d	0.32 ^{ef}	0.13 ^{ab}
2.00 (Control 2)		73.33 ^{cd}	3.20 ^a	3.95 ^c	80.00 ^{ab}	1.20 ^c	1.44 ^{bcd}	0.27 ^{jhi}	0.11 ^{cd}
2.00	0.01	73.33 ^{cd}	2.80 ^{ab}	3.98 ^c	80.00 ^{ab}	1.20 ^c	0.44 ^d	0.26 ^{hi}	0.11 ^{bc}
2.00	0.10	46.66 ^{fj}	3.00 ^a	3.71 ^c	40.00 ^{ab}	1.00 ^c	1.00 ^{cd}	fa	0.11 ^{bc}
2.00	0.15	93.33 ^a	3.00 ^a	3.85 ^c	20.00 ^{ab}	0.40 ^c	0.10 ^d	0.26 ^{hij}	0.11 ^{cd}
3.00 (Control 3)		93.33 ^a	2.20 ^b	3.66 ^c	100.00 ^a	1.00 ^c	2.44 ^b	0.22 ^j	0.09 ^e
3.00	0.01	80.00 ^{bc}	3.00 ^a	3.77 ^c	80.00 ^{ab}	0.80 ^c	0.49 ^{cd}	0.29 ^{fjh}	0.11 ^{bc}
3.00	0.10	86.39 ^b	3.00 ^a	3.71 ^c	40.00 ^{ab}	1.00 ^c	0.11 ^d	0.25 ^{hij}	0.09 ^e
3.00	0.15	80.00 ^{bc}	3.00 ^a	3.51 ^c	40.00 ^{ab}	1.40 ^c	0.17 ^d	0.29 ^{fjh}	0.11 ^{bc}
4.00 (Control 4)		66.26 ^{de}	3.00 ^a	4.15 ^c	40.00 ^{ab}	0.40 ^c	0.26 ^d	0.27 ^{jhi}	0.10 ^{cde}

4.00	0.01	80.00 ^{bc}	2.60 ^{ab}	3.50 ^c	80.00 ^{ab}	1.00 ^c	0.56 ^{cd}	0.23 ^{ij}	0.09 ^c
4.00	0.10	40.00 ^j	3.00 ^a	3.99 ^c	60.00 ^{ab}	1.80 ^c	0.19 ^d	0.32 ^{ef}	0.09 ^c
4.00	0.15	73.33 ^{cd}	2.60 ^{ab}	3.06 ^c	0.00 ^b	0.00 ^c	0.00 ^d	0.36 ^{de}	0.11 ^{cd}
Control 5-MS medium		80.00 ^{bc}	3.00 ^a	9.26 ^a	100.00 ^a	5.80 ^b	4.07 ^a	0.53 ^c	0.09 ^c
Control 6	0.01	65.26 ^c	3.00 ^a	9.42 ^a	100.00 ^a	10.00 ^a	1.90 ^{bc}	0.64 ^a	0.11 ^{bc}
Control 7	0.10	73.12 ^{cd}	2.80 ^{ab}	5.91 ^b	100.00 ^a	10.20 ^a	0.68 ^{cd}	0.60 ^b	0.11 ^{cd}
Control 8	0.15	52.59 ^f	2.80 ^{ab}	6.13 ^b	100.00 ^a	5.80 ^b	0.93 ^{cd}	0.53 ^c	0.09 ^{de}

**Values showed in same column by different letters are statistically different using Tukeys'b Test at 0.01 level of significance

*Values showed in same column by different letters are statistically different using Tukeys'b Test at 0.05 level of significance.

All values are means of 100 explants repeated 4 times, All means for fresh and dry weigh are means of 5 explants repeated 4 times

Control 1 = MS mdium having 1 mg/L BA, Control 2 = MS mdium having 2 mg/L BA, Control 3 = MS mdium having 3 mg/L BA, Control 4 = MS mdium having 4 mg/L BA, Control 5 = MS mdium, Control 6 = MS mdium having 0.01 mg/L NAA, Control 7 = MS mdium having 0.10 mg/L NAA, Control 8 = MS mdium having 0.15 mg/L NAA

DISCUSSION

Although, Turkey is water locked on three sides, most of the Anatolian lands face problem of water shortage and as such could be counted as semi arid. Above described scenario necessitates development of technologies for low water cultivation techniques for rice planting in agreement with Pandey *et al.* (1998), Liu *et al.* 2014a,b). This study reports and compare useful and harmful effects of rice cv. Hamzadere and Osmancik 97 seed priming behaviour with BA, BA+NAA and NAA for 14 days. It may be mentioned that 6-benzylaminopurine (BA) a first-generation synthetic cytokinin, that accelerate growth and division of plant cells leading to emergence of lateral buds, shoot formation and flowering etc. (Sun and Zhang, 2006; Ohashi *et al.*, 2009).

It is also known that NAA is aromatic in structure and stimulate cell elongation, division, and subsequently leads to increase in shoot length, and photosynthesis. NAA is also involved in induction of flowers, setting of fruits, late senescence. They also interfere and suppress bud sprouting (Basuchaudhuri, 2016).

This study has proved that availability of MS medium macro and micronutrients along with vitamins +30 g/l sucrose) are more important compared to the availability of hormones in the form of auxins and cytokinins for growth and development of rice seedlings irrespective of their concentrations and combinations. Marked and significantly different behavior was sighted on germinated seedlings and growth parameters as was realized for each type of treatment. The results indicated that performance of BA, NAA or BA+ NAA primed rice seeds showed inhibition in germination compared to the rice seeds primed with MS medium used singly. It appeared as if MS medium in agar solidified substrate might have stimulated seedling growth in the presence of 30 g/l sucrose. MS medium used singly seems adequate to obtain satisfactory seedling growth in agar solidified MS medium. The results further showed that MS medium

was the best priming medium followed by very low concentration (0.01 mg/L) of NAA.

The results confirmed that there seemed little to be gained, when NAA is used above 0.1 mg/L. Any concentration of NAA exceeding this concentration used singly was partially inhibitory, BA used singly was inhibitory and some concentrations of BA+NAA used in combination was both inhibitory and deteriorative and most of them did not allow root induction on the seedlings. The results of the study are not in agreement with Turetskaya and Polikarpova (1968); Christov and Koleva (1995); who have reported increased root initiation on cuttings of some plants when plant growth regulators were supplemented with vitamin C, B1 or K. The results of this study are also in agreement with De Klerk *et al.* (1997); Rani *et al.* (2008). They have suggested use of MS basal salt mixture and vitamins for in vitro rooting studies.

Contrarily, previous reports suggest positive effects of NAA used singly on growth and development of some monocotyledonous and dicotyledonous plant species. Singh and Sharma, (1982 and and Bangal *et al.*, (1983), have shown that increase in seed and pod weight occurs with NAA foliar application at flowering stage in groundnut, chickpea and faba beans. Khanzada *et al.* (2002) observed maximum number of seeds per pod when NAA was applied 15 days post emergence. Similarly, favourable influence of NAA has been reported for many cereals on increasing yield of cereals like wheat (Alam, *et al.*, 2002; Zahir *et al.*, 1998; Muthukumar, *et al.*,2005). Improvement in rice seed yield of cv. BRRIdhan-29 and BRRIdhan-50 was realized after spray of 200 mg/L NAA during winter season by Golam Adam *et al.*, (2011); and Bakhsh, *et al.* (2011). Root length, root volume and root weight (dry and fresh) were found to show marked increase using 100 mg/L (ppm) of NAA under flooded conditions. Maximum root length, and root weight (fresh and dry) was obtained from 100 mg/L (ppm) NAA. (Sarker *et al.*, 2013). Contrarily, multiplication of rice cells was maximum and positive after priming with agar semi

solidified MS medium that invigorated germination and other parameters positively compared to the seeds that were primed using 0.01, 0.10 and 0.15 mg/L concentrations of NAA.

Chaudhuri *et al.* (1980) and Sarker *et al.* (2013) observed that NAA application had positive effects on total dry matter production at harvest on rice plant fresh and dry weight.

After auxin precursor biosynthesis generation using (Shikimate pathway) in plastids located in cytosols. They conjugate and degrade after reacting with amino acids and sugars. Their subsequent hydrolysis play role in development and growth of plants. As such NAA treatment at balanced or appropriate rate could help in induction and improved activities of adventitious roots. This improves nutrient uptake followed by better cell division and elongation of plants with delayed ageing or senescence as was observed in this study in agreement with Zhi-Guo, *et al.* (2012) and Campanoni *et al.*, (2005).

Possible causes of differential seed germination under the influence of BA, BA-NAA and NAA used singly could be due to their differential effects on rates of cell metabolism that accelerated metabolic changes of primed seeds. All of these activities subsequently resulted in markedly different seed germination and growth rates. The results of the study are in agreement with Liu *et al.*, (2001), who reported positive effects of benzylaminopurine on tillering using low tillering rice cultivar North Rose and high tillering cv Sasanishiki under hydroponic conditions after foliar spray of BA at 6-leaf stage. Foliar BA spray inhibited production of new leaves. Likewise, treating BA singly was better compared to combined treatment effect of BA+NAA. These effects never surpassed plant growth regulators priming behaviour; when MS medium was used singly or MS medium contained any concentration of NAA. Overall, BA or BA+NAA applications were inhibitive to all growth parameters pertaining to cv. Hamzadere and Osmancik 97 in the present study.

Conclusion: Right choice of priming agent could help in improving seed germination and in turn yields. Variable effects on growth components were realized on growth parameters depending on concentrations and combinations of cytokinins or auxins used in the study. It could be implied that priming with MS medium was the best with maximum germination, production of tillers affecting all growth parameters positively including fresh and dry weight followed by priming with 0.01 mg/L NAA in MS medium. Application of this technology could help in better emergence, tillering and establishment compared to all other treatments in rice breeding programs without risk of change of genetic makeup of plants using plant growth regulators. This will also help in preserving agronomically important properties of the selected materials.

REFERENCES

- Ahmad, M.S.A., F., Javed and M. Ashraf (2007). Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa*. L) genotypes. *Plant Growth Regul.* 53:53–63.
- Alam, S.M., A. Shereen and M. Khan (2002). Growth response of wheat cultivars to naphthalene acetic acid (NAA) and ethrel. *Pakistan J. Bot.* 34: 135–137.
- Benderradji, L., F. Brini, K. Kellou, N., Ykhelf, A., Djekoun, K. Masmoudi, H. Bouzerour (2012) Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum aestivum* L.) genotypes under saline and heat stress conditions. *ISRN Agronomy*. 2012: Article ID 367851, 8 pages
- Bakhsh, I., I. Awan, M. Sadiq, M. Niamatuallah and K.U. Zaman (2011). Effect of plant growth regulator application at different growth stages on economical yield potential of coarse rice (*Oryza sativa* L.). *The J. Anim. Plant Sci.* 21: 612–616.
- Bangal, D.B., S.N. Deshmukh and V.A. Patil (1983). Contribution of pod wall in grain development of chickpea (*Cicer arietinum* L.) as influenced by foliar application of growth regulators and urea. *Indian J. Plant Physiol.* 26: 292–295.
- Basuchaudhuri, P (2016). 1-Naphthaleneacetic acid in rice cultivation. *Current Sci.* 110: (1): 10
- Campanoni, P. and P. Nick (2005). Auxin-dependent cell division and cell elongation: 1-naphthalene acetic acid and 2,4-Dichlorophenoxy acetic acid activate different pathways. *Plant Physiol.* 137: 939–948.
- Chaudhuri, D., P. Basuchaudhuri and D.K. Das Gupta (1980). Effect of growth substances on growth and yield of rice. *Indian Agric.* 24: 169–175.
- Faostat 2016. [Faostat.fao.org](http://faostat.fao.org) (accessed 10.07.2016.).
- Farooq M., K.H.M. Siddique, H. Rehman, T. Aziz, D.J. Lee, A. Wahid (2011). Rice direct seeding: experiences, challenges and opportunities. *Soil Tillage Res.* 111, 87-98.
- Golam Adam, A.M.M. and N. Jahan (2011). Effects of naphthalene acetic acid on yield attributes and yield of two varieties of rice (*Oryza sativa* L.). *Bangladesh J. Bot.*, 40: 97–100.
- Harris, D., A.K. Pathan, P. Gothkar, A. Joshi, W. Chivasa and P. Nyamudeza (2001a). On-farm seed priming: using participatory methods to revive and refine a key technology. *Agricultural Sys.* 69: 151-164.
- Harris, D., B.S. Raghuwanshi, J.S. Gangwar, S.C. Singh, K.D. Joshi, A. Rashid and P.A. Hollington (2001b). Participatory evaluation by farmers of

- 'on-farm' seed priming in wheat in India, Nepal and Pakistan. *Exp. Agric.* 37: 403-415.
- Harris, D., A. Mottram (2005) Practical hydration of seeds of tropical crops: 'On-arm' seed priming. In: *Handbook of Seed Science and Technology* (A. S. Basra, Ed.), The Harworth Press, New York. pp. 724-734.
- Harris, D., R.S. Tripathi and A. Joshi (2000). On farm priming to improve crop establishment and yield in direct seeded rice in IRRI pp.164. International Workshop on Dry Seeded Rice Technology, held in Bangkok, 25-28 January 2000. The International Rice Research Institute. Manila. The Philippines.
- Anonymous (2016b). <http://www.bassel.com.tr/?p=41> (accessed 10.07.2016)
- Anonymous (2016a). http://www.tekcantohum.com/celtik-cesitleri_hamzadere.html (accessed 10.07.2016)
- Anonymous (2016c). http://www.tray-fa.com/index.php?option=com_content&task=view&id=70&Itemid=32 (accessed 31.12.2014).
- Khanzada, A., M. Jamal, M.S. Baloch and K. Nawab (2002). Effect of naphthalene acetic acid (NAA) on yield of soybean. *Pakistan J. Boil. Sci.* 3: 856-857.
- Ladha, J.K. and P.M. Reddy (2003) Nitrogen fixation in rice systems: state of knowledge and future prospects. *Plant Soil* 252: 151. doi: 10.1023/A:1024175307238
- Liu, H., H. Saddam, M. Zheng, S. Penf., J. Huang, K. Cui and L. Nie (2014a). Dry direct-seeded rice as an alternative to transplanted-flooded rice in Central China. *Agron. Sustain. Dev.* DOI: 10.1007/s 13593-014-0239-0 (2014).
- Liu, H., H. Saddam, M. Zheng, L. Sun, F. Shah, J. Huang, K. Cui and L. Nie (2014b). Progress and constraints of dry direct-seeded rice in China. *J. Food Agric. Environ.* 12: 465-472.
- Liu, Z., Y. Goto, I., Nishiyama and M. Kokubun (2001). Effects of Foliar and Root-Applied Benzylaminopurine on tillering of rice plants grown in hydroponics. *Plant Prod. Sci.* 4(3) : 220-226.
- Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.* 15(3) : 473-497.
- Muthukumar, V.B., K. Vebyudham and N. Thavaprakash (2005). Growth and yield of baby corn (*Zea mays* L.) as influenced by GPRs and different times of nitrogen application. *Res. J. Agric. Biol. Sci.* 1: 303-307.
- Ohashi, F., S. Ueda, T. Taguri, S. Kawachi and H. Abe (2009). Antimicrobial activity and thermostability of silver 6-benzylaminopurine montmorillonite. *Appl. Clay Sci.* 46, 296-299.
- Pe'rez-Clemente RM, Go'mez-cadenas A (2012) In vitro tissue culture, a tool for the study and breeding of plants subjected to abiotic stress conditions. In Leva A, Rinaldi MRR (eds), *Recent advances in plant in vitro culture* (pp 92-108). ISBN: 978-953- 51-0787-3
- Snedecor, G.W. and W.G. Cochran (1989). *Statistical Methods*, Eighth Edition, Iowa State University Press, Iowa.
- Pandey, S. and L.E. Velasco (1998). Economics of direct seeded rice in Illoilo: lessons from nearly two decades of adoption. *Social Science Division Discussion Paper*. Manila (Philippines): International Rice Research Institute.
- Rengasamy, P (2002) Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. *Aust. J. Exp. Agr.* 42:351-361.
- Sarker, B.C., B. Roy, R. Fancy, W. Rahaman and S. Jalal (2013). Response of root growth and yield of rice (BRRI dhan-28) under different irrigation frequencies and plant growth regulator. *J. Sci. Technol.* 11: 51-55.
- Basuchaudhuri, P (2016). *Nitrogen Metabolism in Rice* CRC Press, Boca Raton, Florida.
- Turetskaya, R., and F. Polikarpova (1968). Vegetativnoje razmnoženije rastenij s primenenijem stimulatorov rosta. Moskva, Izdatel'stvo Nauka: 93. [In Russian]
- Christov, C., and A. Koleva (1995). Stimulation of root initiation in hardwood sweet and sour cherry rootstocks (*Prunus mahaleb* L.). *Bulgar. J. Pl. Physiol.* 21(1): 68-72.
- Singh, G.S. and B. Sharma (1982). Effect of plant growth regulators on groundnut productivity. *Indian J. Ecol.* 12: 267-272.
- Small, E (2009). *Rice. Top 100 Food Plants*. NRC Research Press, Ottawa.
- Sun, D. and H. Zhang (2006). Voltammetric determination of 6- benzylaminopurine (6-BA) using an acetylene black-dihexadecyl hydrogen phosphate composite film coated glassy carbon electrode. *Anal. Chim. Acta.* 557: 64-69.
- Watanabe, T. and K. Sogawa (1994). Growth and yield analysis of rice plants infested with long-distance migratory rice plant hoppers. III. Quantitative evaluations of vegetative growth of rice plants infested white-backed planthopper, *Sogatella furcifera* Horva'th (Homoptera: Delphacidae). *Jpn. J. Appl. Entomol. Zool.* 38: 275-281.
- Watanabe, T., L.T. Fabellar, L.P. Almazan, E.G. Rubia, K.L. Heong and K. Sogawa (1997). Quantitative evaluation of growth and yield of rice plants infested with rice plant hoppers. pp. 365-382. In M.J. Kropff *et al.* (eds.) *Applications of Systems*

- Approaches at the Field Level. Kluwer Academic Publishers, Dordrecht.
- De Klerk, G.J., J. Ter Brugge and S. Marinova (1997). Effectiveness of indoleacetic acid, indolebutyric acid and naphthalene acetic acid during adventitious root formation *in vitro* in Malus 'Jork 9'. Plant Cell, Tissue and Organ Culture, 49: 39–44.
- Rani, A.S., B.J. Lakshmi and K.J. Reddy (2008). In vitro regeneration from nodal explants in an exotic medicinal tree *Tabebuia aurea* (Manso) Benth. & Hook. & ex. S. Moore. Advances in Plant Sciences, 21 (2): 373–376.
- Zahir, Z.A., A. Rahman, N. Asgar and M. Arshad (1998). Effect of an auxin precursor L-tryptophan on growth and yield of rice. Pakistan J. Biol. Sci. 1: 354–356.
- Zhi-Guo, E., G. Lei, W. Lei (2012). Molecular mechanism of adventitious root formation in rice. Plant Growth Regul. 68: 325–331.