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Physiological role of yeast extract and nicotinamide on *Pisum sativum* L. plants under heat stress

Mervat Sh Sadak*

Botany Dept, National Research Centre, 33 El-Tahrir St., Dokki, 12622 Giza, Egypt

Abstract : Two pot experiments were carried out during two successive seasons and sowing at two dates, early date and normal date to study the effect of early sowing date at high temperature and presoaking in yeast extract (0.1%, 0.2% and 0.3%) and nicotinamide (2.5, 5.0 and 7.5 mg/l) on growth, yield components and some biochemical aspects of *Pisum sativum* L. plant. The early date of sowing at high temperature in general caused significant decreases in shoot length, number of shoot and leaves per plant, fresh and dry weights of shoots. Also, high temperature caused reductions in yield components (number of pods/plant, pods wt/plant, seeds wt/pod, seeds wt /plant and 100 seeds wt), photosynthetic pigments and indole acetic acid (IAA) content concomitantly with increases in total soluble sugars (TSS), free amino acids, proline and phenolic contents. Soaking of *Pisum sativum* L. seeds in yeast extract or nicotinamide at the two date of sowing significantly increased growth and yield components concomitantly with increases in the amounts of IAA, photosynthetic pigments. Moreover, these treatments caused more significant increases in TSS, free amino acids, proline and phenolic contents.

Key words: Heat stress, IAA, Nicotinamide, Pea, TSS and Yeast extract.

Introduction

In Egypt, one of the most important vegetable leguminous crop is pea (*Pisum sativum* L.) plant that is growing during winter season for local consumption and for export. Due to its high nutritional values of seeds, pea a winter legume crop plays an important role in human nutrition as it considered as a source of protein, minerals and different vitamins.

Environment is an important factor that play significant role in growth of plant and development. Atmospheric temperature changes are often very sudden and different plants cannot adjust to these changes and can damaged beyond recovery¹. High temperature that cause heat stress may increase and induce evaporative demand of plant and hence indirectly contribute to water deficiency or salt stress. In addition, high temperature could alter membrane permeability and transpiration rate of plant cells leading to reduction of growth and finally poor yield. At high temperature various physiological and biochemical activities decline as a result of inactivation of enzymes and other proteins. Earlier studies on different plant species have showed the effect caused by high temperature on changing endogenous levels of natural plant growth regulators².

Higher temperature stress either accelerates the formation of toxic reactive oxygen species (ROS), i.e., H₂O₂, OH⁻, O⁻² levels within plant tissues or impairs the normal defense mechanisms against ROS toxic effects. ROSs is toxic, highly reactive and can lead to the oxidative destruction of cells. The consequences of ROSs formation depend on the intensity of stress and on the physicochemical conditions in the cell. It has been

generally accepted that ROSs produced under stress are the decisive factor that causes lipid peroxidation, enzyme inactivation³, etc. . In normal conditions, lipid peroxidation is a natural metabolic process. Lipid peroxidation activation is one of the possible results of a rapid response to stress. One of lipid peroxidation products MDA was investigated in the present work. The content of MDA is often used as an indicator of lipid peroxidation resulting from oxidative stress⁴. Moreover, ROS damage chloroplast, reduce carbohydrate synthesis and exportation, attack cell membranes, led to their degradation and leakage of cell solutes, denaturation of proteins and enzymes, damage of nucleic acids, degradation of chlorophyll and suppression of all metabolic processes, and finally senescence and death of cells and tissues^{5&6}. One of the protective mechanisms of plant include accumulation of some compatible solutes such as proline and total soluble sugars.

So, amelioration of adverse effects of high temperature or heat stress by using natural and safety substances that are rich sources of phytohormones to improve tolerance of plants, growth and productivity has gained a great attention nowadays⁷. Yeast is considered a natural safety biofertilizer and rich source of phytohormones (especially cytokinins), sugars, vitamins (as vitamin B or nicotinamide) enzymes, amino acids and minerals⁸. It is well known that the improving effect of yeast could be attributed to its enhancement role on production of some phytohormones, nutrient uptake improvement, increasing enzyme activity, convert insoluble forms of phosphorus into soluble one for improving availability of phosphorus to plant all these lead to increased growth of plant. Also, yeast treatment to plant increase release of CO₂ that reflected on improving net photosynthesis⁹.

Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. It, also known as niacinamide and nicotinic acid amide, is the amide of nicotinic acid (vitamin B3/niacin). Nicotinamide is a well-characterized constituent of the pyridine dinucleotide coenzymes NADH & NADPH, which are involved in many enzymatic oxidations - reductions reactions in living cells. In addition, nicotinamide is a stress-associated compound that induces and regulates secondary metabolic accumulation and/or the manifestation of defense metabolism in plants¹⁰. Nicotinamide is a well-characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH, it is contributory in many enzymatic systems. Nicotinamide is efficiently and effectively taken up by plant tissue cultures¹¹.

So the aim of this investigation is to study the physiological role of yeast extract and nicotinamide as seed soaking treatment on improvement of thermotolerance of pea plant via improving some physiological and biochemical parameters.

Materials and Methods

The experimental materials used in this investigation were the seeds of pea (*Pisum sativum* L. Master B) provided from the Agricultural Research Centre, Giza, Egypt. Two pots experiments were carried out at the screen of National Research Centre, Egypt. In the first experiment seeds were sown on 3rd September at temperature ranged between 38-32°C / 25-22°C day/night, the seeds of the second experiment were sown on November 3rd at temperature ranged between 26-23°C / 22-16°C day/night during two successive seasons 2012 and 2013. The natural growth substances used were yeast extract and nicotinamide.

A homogeneous lots of pea seeds were divided into 7 groups. The first healthy group were soaked in tap water to serve as control, while the seeds of other groups were soaked separately in aqueous solutions of three concentrations of yeast extract namely (0.1%, 0.2% and 0.3%) and three concentrations of nicotinamide namely (2.5, 5.0 and 7.5 mg/l). Soaking of seeds in tap water as well as different concentrations of yeast extract and nicotinamide were carried out at 25°C for 12 hours with aeration of the solutions. Then seeds were air dried.

The soaked seeds then were sown in pots (30 cm in diameter) containing equal amounts of clay and sandy soils in the ratio 2:1. The pots were distributed in a complete randomized design. 10 Seeds were sown in each pot. Thinning was done after 15 days so that 5 uniform seedlings per pot were left for experimentation. At 30 days old plant samples were taken to measure some growth parameters, indole acetic acid, photosynthetic pigments in fresh leaves. After drying leaves, total soluble sugars, free amino acids, proline and phenolic contents. At maturity of plants parameters of yield components were measured at 60 – 70 days old plants.

Chemical analysis:

Photosynthetic pigments (Chlorophyll a, chlorophyll b and carotenoids concentrations) were estimated using the method¹². Indole acetic acid was determined according to the method reported¹³. Total soluble sugars (TSS) were extracted¹⁴ and were analyzed¹⁵. Free amino acid content was extracted according to the method described¹⁶. Free amino acid was determined with the ninhydrin reagent method¹⁷. Proline was assayed according to the method described¹⁸. Total phenolic compounds were determined according to the method¹⁹.

Statistical analysis

The data were statistically analyzed on complete randomized design system according²⁰. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Results

Growth responses and yield attributes:

Growth responses of *Pisum sativum* L. plants to varying sowing dates and soaking in yeast extract and nicotinamide different concentrations before sowing are presented in Table (1). Sowing pea plants at high temperature by early sowing caused significant reductions in growth parameters (shoot length (cm), branches number/plant, leaves number/plant, shoot fresh and dry weight /plant(g)). The percentage of decreases were 22% in shoot length, 60% in branches number/plant, 17% in leaves number/plant, 39% in shoot fresh wt and 23% in shoot dry wt. With respect to the effect of soaking pea seeds in different concentrations of yeast extract and nicotinamide prior to sowing, data presented in Table (1) show that, different concentrations of yeast extract or nicotinamide caused significant increases in different studied growth parameters. Table (1) also show that yeast extract treatments were more effective than nicotinamide treatments as it caused more increases in growth parameters. The most effective treatment was 0.2% yeast extract as it caused the highest increases in shoot length, branches & leaves / plant, fresh & dry weights of shoots either of early sowing or normal sowing plants.

Table (1): Effect of yeast extract and nicotineamide on growth parameters of *Pisum sativum* L. at two dates of planting (Data are means of two seasons).

Date of sowing	Treatment	Conc	Shoot length (cm)	Branches no/plant	Leaves no/plant	Shoot fresh wt (g)	Shoot dry wt (g)
Early sowing	Yeast extract	0	17.50	1.00	7.25	2.25	0.32
		0.1%	18.25	1.25	8.25	3.47	0.45
		0.2%	20.15	2.00	8.75	4.75	0.62
		0.3%	18.50	1.75	8.00	4.35	0.51
	Nicotinamide	2.5 mg/l	17.25	1.25	8.00	3.41	0.42
		5.0 mg/l	19.75	1.75	8.50	4.36	0.57
		7.5mg/l	18.25	1.75	8.00	4.12	0.51
Normal sowing	Yeast extract	0	22.50	2.50	8.75	3.73	0.42
		0.1%	25.50	2.75	9.50	4.39	0.49
		0.2%	28.25	3.25	11.25	5.48	0.72
		0.3%	26.50	3.00	10.25	5.01	0.63
	Nicotinamide	2.5 mg/l	24.50	2.50	9.50	4.16	0.47
		5.0 mg/l	27.50	3.00	10.75	5.42	0.59
		7.5mg/l	26.50	2.75	10.25	5.14	0.62
LSD at 5%			1.021	0.213	0.985	1.023	0.014

Data presented in Table (2) show the effect of different sowing dates on yield components of pea plants. It is clear that, the number of pods/plant, pods wt/plant, seeds wt/pod, seeds wt /plant and 100 seeds wt were decreased in plants sowed at early date compared with those plants sowing at delaying date. The percentages of reductions were 54% of number of pods/plant, 48% of pods wt/plant, 55% of seeds wt/pod, 68% of seeds wt /plant and 43% of 100 seeds wt. On the other hand, presowing pea seeds with different concentrations of yeast extract and nicotinamide could alleviate the reduced effect of high temperature of early date by increasing the different yield components. Moreover, different treatments increased yield and yield components of pea plant at normal date of sowing as compared with untreated control plant.

Table (2): Effect of yeast extract and nicotinamide on yield components of *Pisum sativum* L. at two dates of planting (Data are means of two seasons).

Date of sowing	Treatment	Conc	Pods no/plant	Pods wt/pant	Seeds no/pod	Seeds wt/pod	Seeds wt/plant	100 Seeds wt
Early sowing	Yeast extract	0	3.5	15.26	3.25	1.59	5.565	22.26
		0.1%	4.25	17.45	4.75	2.74	8.645	28.58
		0.2%	5.75	25.25	5.75	3.42	11.665	32.66
		0.3%	5	21.42	4.75	3.41	10.05	29.2
	Nicotinamide	2.5 mg/l	4.25	16.4	4.5	2.54	7.795	26.18
		5.0 mg/l	5.5	23.5	5.25	3.41	9.755	29.02
		7.5mg/l	5	20.35	4.75	3.18	8.9	25.6
Normal sowing	Yeast extract	0	7.75	29.85	6.25	3.54	17.435	39.74
		0.1%	8.25	35.25	7.24	4.75	20.1875	43.75
		0.2%	9.25	42.52	8.57	5.24	24.47	52.88
		0.3%	9	37.57	8.25	4.72	19.48	47.92
	Nicotinamide	2.5 mg/l	7.5	33.75	7.01	4.43	19.225	46.9
		5.0 mg/l	8.5	39.78	7.29	5.12	23.52	51.08
		7.5mg/l	8.25	34.54	7.21	5.01	21.3325	45.33
LSD at 5%			0.87	2.47	0.98	0.87	1.42	2.74

Photosynthetic Pigments:

Table (3) show the effect of soaking pea seeds before sowing in different concentrations of yeast extracts or nicotinamide on pea plant sowing at early and normal dates. Data clearly show that seed sowing at early date (at high temperature) induced significant decreases in photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll a+b, carotenoids and consequently total pigments) contents of *pisum sativum* L as compared with the control plants sowing at normal date. On the other hand, treatment of pea seed with different concentrations of yeast extract or nicotinamide increased significantly photosynthetic pigments as compared with untreated control either of plants planted at early date or normal date. 0.2% yeast extract was the most effective treatment followed by 5.0 mg/l nicotinamide on increasing the photosynthetic contents.

Table (3): Effect of yeast extract and nicotineamide on photosynthetic pigment (mg/100 g FW) of *Pisum sativum* L. at two dates of planting (Data are means of two seasons).

Date of sowing	Treatment	Conc	Chl a	Chl b	Chl a+b	Carotenoids	Total pigments
Early sowing	Yeast extract	0	624	195	819	171	990
		0.1%	742	256	998	212	1210
		0.2%	787	285	1072	241	1313
		0.3%	723	267	990	233	1223
	Nicotinamide	2.5 mg/l	695	243	938	209	1147
		5.0 mg/l	703	270	973	226	1199
		7.5mg/l	713	263	976	219	1195
Normal sowing	Yeast extract	0.0	796	373	1169	229	1398
		0.1%	857	432	1289	268	1557
		0.2%	935	462	1397	298	1695
		0.3%	904	440	1344	236	1580
	Nicotinamide	2.5 mg/l	817	412	1229	238	1467
		5.0 mg/l	914	446	1360	272	1632
		7.5mg/l	872	427	1299	243	1542
LSD at 5%			11.52	8.35	17.36	7.85	18.36

Endogenous indole acetic acid (IAA):

Data presented in Table (4) show the effect of heat stress by early sowing of pea plant and the effect of different concentrations of yeast extract or nicotineamide. It is clearly demonstrated from the results that indole acetic acid (IAA) contents showed significant reduction in shoot tissue of pea plants sowing at early date (at high temperature) as compared with the control pea plants sowing at suitable normal date. On the other hand, presoaking pea seeds in different concentrations of yeast extract or nicotineamide caused significant increases in indole acetic acid contents as compared with untreated controls of both time of sowing (early time and normal time). The most effective treatment was 0.2% yeast extract followed by 5.0 mg/l nicotineamide in both dates of sowing as they cause the highest increases in endogenous IAA content.

Table (4): Effect of yeast extract and nicotineamide on IAA ($\mu\text{g/g}$ FW), TSS, Free amino acids, proline and phenolic contents (mg/g dry wt) of *Pisum sativum* L. at two dates of planting (Data are means of two seasons).

Date of sowing	Treatment	Conc	IAA ($\mu\text{g/g}$ FW)	TSS	Free amino acid	Proline	Phenolics
					(mg/g Dry wt)		
Early sowing	Yeast extract	0	22.54	37.52	127.25	35.35	28.65
		0.1%	36.35	50.52	134.52	47.25	37.25
		0.2%	42.85	59.35	162.52	52.52	42.21
		0.3%	32.45	48.25	142.35	49.35	36.52
	Nicotinamide	2.5 mg/l	29.52	46.25	134.52	37.35	32.52
		5.0 mg/l	38.54	49.25	157.25	50.24	40.00
		7.5 mg/l	32.04	47.25	152.25	44.25	34.25
Normal sowing	Yeast extract	0.0	34.25	28.96	112.52	24.25	18.35
		0.1%	39.86	35.42	138.35	33.52	20.52
		0.2%	52.62	47.52	145.32	42.52	25.42
		0.3%	48.75	41.35	132.52	32.32	20.14
	Nicotinamide	2.5 mg/l	37.52	33.52	126.35	28.65	20.51
		5.0 mg/l	50.42	46.52	139.65	38.35	23.52
		7.5 mg/l	45.32	32.74	124.52	37.52	20.41
LSD at 5%			2.41	4.23	9.52	5.35	3.54

TSS, Free amino acids, proline and phenolic contents:

It is clearly shown from Table (4) that, seed sowing at early date of planting (at high temperature) increased significantly total soluble sugars (TSS), free amino acids, proline and phenolics contents of pea shoots. Meanwhile, soaking pea seeds in different concentrations of yeast extract or nicotinamide caused more significant increases in TSS, free amino acids, proline and phenolics of pea plants as compared with those untreated plants either at early date of sowing (heat stress) or normal date of sowing (Table 4). 0.2 % yeast extract treatment was the most effective treatment followed by 5.0 mg/l nicotinamide treatment.

Discussion

Growth responses and yield attributes:

Data of growth responses of *Pisum sativum* L. plants to varying sowing dates and soaking in yeast extract and nicotineamide different concentrations before sowing in Table (1) show that, sowing pea plants at high temperature by early sowing caused significant reductions in growth parameters (shoot length (cm), branches number/plant, leaves number/plant, shoot fresh and dry weight /plant). These results are supported with the finding^{21,22,2& 23} on wheat, rice and wheat plant. These reductions in growth parameters of pea plants by early sowing mainly resulted from marked reduction in their growth duration caused by relatively higher temperature prevailing during the critical growth phases of early sowing pea plants. In addition, the reduction in the studied growth parameters of pea plant in response to early sowing date can be ascribed to the effect of high temperature on the membrane permeability and the transpiration rate²⁴. With regard to the effect of yeast extract, soaking pea seeds in different yeast extract and nicotineamide concentrations, data show that different treatments increased significantly all the studied growth parameters of pea plants either at early and normal dates of sowing (Table 1). These results of yeast extract are similar to those obtained earlier²⁵ on soybean. The enhancement of pea plant growth in response to exogenous treatment with yeast extract might be attributed to its contents of growth regulators, vitamins (especially vitamin B), sugars, macro and micro nutrients²⁶ and relatively large amount of free amino acids and short peptides of two or three amino acids as well as long chain protein hydrolysates²⁷. As well as, Wanas²⁸ and El- Sherbeny et al²⁹ attributed the enhancement effect of yeast to the stimulation of photosynthetic pigments and improvement of enzyme activity and photosynthetic process that in turn encourage vegetative growth of broad bean. The enhancement role of nicotineamide on pea plant either at normal or early date of sowing are in accordance to those results obtained^{30&31} on sunflower and faba bean. The improving role of nicotineamide may attributed to its role as growth stimulants which can play a role in mitigating the adverse effect of heat on metabolic activities relevant to growth through increasing IAA content (Table 4). These were further corroborated by the significantly higher levels of carbohydrates observed generally in the test plants nicotineamide treated³⁰.

Table (2) show the effect of early sowing date on pea plant. Early sowing date caused significant reductions in yield and yield components of pea plants as compared with those planted at normal date. These results are in agreement with those obtained by many investigators^{31,2,32,33&34} by different stresses on different plant species. The depressive effect of high temperature on yield and yield components might be attributed to the inhibitory effects of heat on vegetative growth (Table 1), chemical composition of plant, reduced endogenous indole acetic acid (IAA) (Table 4). Such variation were reflected on yield production. Concerning the stimulative role of yeast extract on yield, many investigators^{25,35&36} confirmed our obtained results on soybean and apricot plants and attributed this result to the stimulating role on plant building up dry matters and enhanced orientation and translocation of photoassimilates from leaves (source) to flowers and seeds (sink). In addition, yeast extract can play a beneficial role in enhancing flower formation and their set of some plants and improved the accumulation of carbohydrate due to its high auxin and cytokinin contents³⁷. With respect to nicotineamide effect our obtained results are in accordance with those obtained earlier^{30&31} on sunflower and faba bean plants under stress. Nicotinamide vitamin appear to form sink mobilizing different nutrients which are involved in building new tissues in pea plants and/or enhance photosynthesis photosynthesis. Moreover, these increases in yield components mainly due to the effect of nicotineamide on enhancing protein synthesis and delaying senescence³⁸.

Photosynthetic pigments:

It is clearly demonstrated in the present investigation that early sowing of seeds at high temperature induced significant decreases in chlorophyll a, chlorophyll b, and carotenoids contents of *Pisum sativum* L. as compared with the control plants sowing at late date (at suitable temperature). These reductions of leaf photosynthetic pigments in pea plants sowing at high temperature might be due to inhibition the biogenesis of pigments. In agreements with this results, it was reported that high temperature greatly affected various physiological functions of crops, thereby, reducing growth rate and photosynthesis^{39&40}. With regard to the stimulative effect of either yeast extract or nicotinamide treatments, our obtained results of yeast extract were confirmed on tomato and soybean plants^{41&.9}. The enhancement role of yeast extract might be attributed to the role of yeast cytokinins in delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis as well as affecting the balance between photosynthesis and photorespiration in plants⁴¹. The enhancement effect of nicotinamide are confirmed by the results obtained on sunflower and faba bean^{30&31}. These effect of nicotinamide may interfere with the protection of chloroplast and their membrane and the maintaining their integrity or vitamins protect chloroplast from oxidative damage⁴³. In addition, nicotinamide has a role in activation of enzymes that regulate photosynthetic carbon reduction⁴⁴.

Endogenous indole acetic acid:

Heat stress usually results in deep observed metabolic changes, which are reflected by disturbed concentrations of phytohormones and further on by altered ratios between them²⁴. It is clearly demonstrated from Table (4) that, IAA content showed a reduction in shoot tissue of early treated plants as compared with those plants normal date of sowing. These results indicate that high temperature severely inhibits the biosynthesis of auxins (IAA) and/or increase their degradation or transformation into inactive form. In response to the effect of presoaking pea seeds in different concentration of yeast extract or nicotinamide, different concentrations of yeast extract increased IAA contents of pea shoot tissues as compared to untreated plants in both dates of sowing (Table 4). These increases in IAA contents are parallel with the increases in growth rate (Table 1). These increases in response to yeast extract treatment might be due to thar yeast extract is enrich source of phytohormones especially IAA²⁵.

TSS, free amino acids, proline and phenolic contents:

It is clearly shown from Table (4) that, seed sowing at early date of planting (at high temperature) increased significantly total soluble sugars (TSS), free amino acids, proline and phenolics contents of pea shoots. These results are in concurrent with the results of stress effect on TSS, free amino acids and proline contents of different plant species obtained by different investigators⁴⁴⁻⁴⁶ on different plant species. These results indicated that these increases in TSS, free amino acids, proline and phenolics contents at high temperature might be one of the earliest metabolic responses triggered in transduction pathways that links the perception of many environmental stress to the elicitation of physiological responses at cellular levels⁴⁵. Meanwhile, soaking pea seeds in different concentrations of yeast extract or nicotinamide caused more significant increases in TSS, free amino acids, proline and phenolics of pea plants as compared with those untreated plants either at early date of sowing (heat stress) or normal date of sowing (Table 4). These results may be due to the yeast extract is an enriched source of phytohormones especially IAA, cytokinins, vitamins, enzymes and free amino acids⁴⁷.

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