# Effect of Nutrient Media and Phytohormones on in Vitro Culture Initiation of Juno tratt. Species

**Elena Nikitina, Komiljon Tojibaev, Hulkar Halbekova**

***Abstract:-*** ***A long period of dormancy in seeds delay plant breeding. Using embryo culture technique, the breeding cycle can be shortened in plants. The aim of this experiment was breaking the seeds dormancy of Juno Tratt. species due to their structure peculiarities. The method of seed germination in vitro was appliedin order to accelerate plants reproduction and to obtain young and surface sterile plant material. Therefore, the calculation of optimum nutrient media for plant regeneration was conducted to provide maximum growth and development of explants and quality of developed plantlets. This work emphasizes on optimization of nutrient media. The significant effect of phytohormones (6-benzylaminopurine (BAP), indolyl-3-acetic acid (IAA), kinetin) at different concentrations and combinations during organ culture has been shown.***

***Keywords: Juno warleyensis, Juno vicaria, in vitro, growth regulators, culture medium, seed germination, rooting.***

## I. INTRODUCTION

Flora of Uzbekistan is characterized by the plenty of ornamental plants species. Among them a special place is occupied by bulbous and rootlike plants, which are the relatives of some beautiful species of Iridaceaefamily. It includes a specific group of relic endemic plants, which are nearly to disappear from the earth's surface. There is a huge risk to lose a unique species and their genetic information.Many irises of the Juno section are ornamental flowers that have been in cultivation for a long time for their beautiful flowers.

*Juno Tratt.* is the original group of plants in morphological, systematic, phylogenetic, biological, ecological, geographical and other properties. They form a natural group characterized by their bulbs which are somewhat fleshy and have from a few to several thick, also fleshy storage roots (Rodionenko G.I., 1961).

The bulbous group of irises, the junos has been treated taxonomically by various authors as subgenus *Scorpiris* Spach. within the genus *Iris* L. (Hall T. et. al., 2000; Ikinici et. al., 2011). It comprising some 57 species at present time (Mathew B., 2000). The most occur from the Middle East to Central Asia, but none quite get to China. Thus, Central Asia is the center of diversity *Juno* representatives. In Uzbekistan distribute at least 36 species, it is more than half of all species of *Juno (Tratt*.) Benth. Et Hook. genus (Khassanov, F.O., Rakhimova, N., 2012). This quantity is updated constantly (Jilek J., 2013; Tojibaev K.Sh.,Turginov O., 2014; Lazkov G.A., Naumenko A.N., 2014.). *Juno* species are valuable rare and endangered ornamental plants. Their number is constantly decreasing due to habitat loss and degradation by various factors. So, they require measures for them protection, preservation and distribution as promising spring ornamental plants.At present time our scientists carried out carefully herbarium specimens (TASH) and field study data in order to adjust the species amount of this genus on Uzbekistan territory (Turginov O. et. al., 2014).

The embryo of *Juno's* mature seeds are surrounded by solid endosperm and thick seed coat. Whereupon, the long physiological dormancy (2-3 years) occurs in seeds, inherent their species. As a result, the seed breeding is difficult. The problem solution can be achieved by use of culture *in vitro* method that in a short period allows obtaining the significant amount of planting material.

The culture *in vitro* significantly reduces the seed dormancy of this genus.The cotyledonary leaf is formed in two weeks under seed germination *in vitro*, afterward the first and the second true leaves. So, the period from the seedling to the immature stage takes 20-25 days, compared to two or three years of ontogenesis period under open ground conditions.Thus, the growing *in vitro* conditions accelerates greatly the germination and seedlings development. It is concluded that junos, as an object, has a great economic potential.

The components of nutritional medium play the main role in the process of introduction into culture stage. The influence of these components can be observed up to one months of cultivation.

## II. MATERIALS AND METHODS

The research objects were *JunoTratt.* species: *Juno warleyensis (Foster) Vved., Juno vicaria Vved.* early spring ephemeroids.

*J. vicaria Vved.* perennial; roots thickened, fusiform; bulb 1 - 3 cm in diameter, well developed; stem 20-40 cm long; leaves pale green. Flowers l-4, inodorous. Perianth tube 4-4.5 cm long, violet; stigmas semicircular, emarginate; anthers and pollen whitish. Areal: grows in river valleys, sand and pit-run fines-debris slopes, stony slopes and among rocks in the lower mountain zone (1100-2900 m above sea level).Endemic. Distribution: South-Western Pamir-Alay-Baysun mountains, Gissar Range, Kugitang. Flowering and fruiting: March-April.

*J. warleyensis (Foster) Vved*. perennial; roots only slightly thickened, fusiform; bulb 2.5 cm in diameter, well developed; stem 20-40 cm long, with distant leaves and

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conspicuous internodes; leaves pale green. Perianth tube 4.5-5 cm long, greenish; stigmas semicircular, emarginate; anthers and pollen whitish. Areal: stony slopes in the lower mountain zone at 1800-2200 m above sea level. Endemic. Distribution: Pamir-Alay- Samarkand mountains to Mogian-darya. Flowering and fruiting: March-April.

According Sikura A.I. (1999) *J.vicaria*, *J.warleyensis*have the largest weight of bulbs and occur in almost all altitudinal belts. Consequently, it can be assumed that these species havewide adaptive capabilities.

The primary explant were mature seeds,collected in Surkhandarya region, Baysuntau, the South-Western spur of the Hissar Range in Uzbekistan.

The junos study *in vitro* was conducted according to scheme1. seeds sterilization; 2. selection of culture media for stimulation of germination; 3. receipt of seedlings; 4. stimulation of root formation.

For germination increasing it is important to choose the pretreatment and culturing conditions. The seeds were stratified for 4 weeks ($+4C^0$) and had shown the best results. Operations at aseptic conditions, preparation and sterilization of nutrient medium were performed according to common techniques (Butenko, 1999). The different sterilizing agents were chosen at present step.

The elaborated sterilization method with high viability let us reach 100% sterility explants. The best antiseptic effect was achieved by the following scheme sterilization in laminar box: 1) treatment with 70% ethyl alcohol - 1 min; 2) washing in sterile distilled water - 10 min; 3) treatment with 0.1% $AgNO_3$ solution - 10 minutes; 4) rinsing the seed in sterile distilled water three times.

On the stages of explants sowing, regenerates cultivation, proliferation, in all variants of this experiment a mineral base of Murashige, Skoog (1962) culture medium (Himedia, India) had been used with agar (0.6%) supplemented with phytohormones. pH of the culture media prior to autoclaving was adjusted to 5.8 with drop wise of 0.5N KOH. The IAA stock solution was prepared by dissolving 10mg of IAA in few drops of 0.5N NaOH. Distilled water was added to make up the solution up to 10ml. The BAP and kinetin stock solution was prepared by dissolving 10 mg of BAP in 95% ethanol and made up to 10ml using distilled water. Final concentrations of both auxin and cytokinin were kept 10mg/10ml. All stock solution was stored in refrigerator. Each explant was inoculated into the freshly prepared culture media. It was then sealed and labeled appropriately and kept inside the culture room maintained at $25C^0$, the light condition 4 thousand luxury, 16/8 hours (light/dark) photoperiod. Cultures were observed daily for eight weeks.

## III. RESULTS AND DISCUSSION

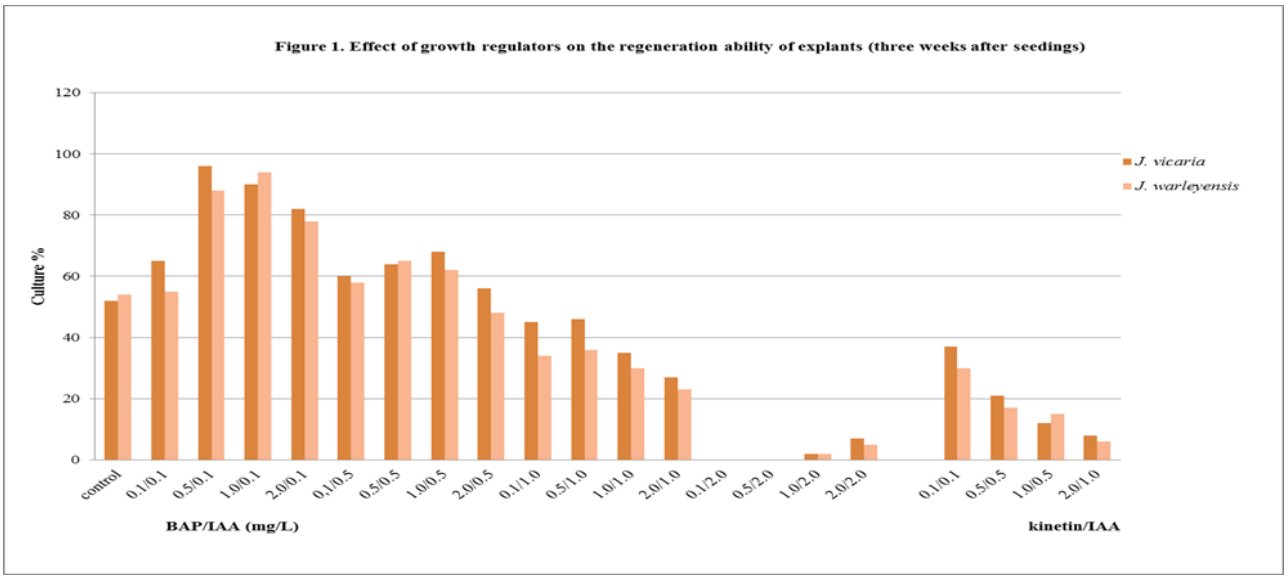
Specificity of introduction in culture is the presence or absence, as well as combination of growth regulators cytokinins and auxins, that depends on species genotype. Due to specific dormancy of studied taxa, individual schemes have been used for each culture. Therefore, the careful selection of the optimum concentration had been done.

Considering mentioned above, a system of stepwise introduction in culture of seeds was developed. The growth regulators were incorporated in culture medium at appropriate concentrations on the step of introduction in culture. It was tested more than a dozen species of hormones in culture media ratios: BAP (0.1, 0.5, 1.0, 2.0 mg/L), kinetin (0.1, 0.5, 1.0, 2.0 mg/L) and different combinations of these agents with IAA (0.1, 0.5, 1.0, 2.0 mg/L).

It should be noted that junons were responsive to the using of growth regulators. Results obtained when analyzed at 5% level of significance showed that the concentration of both hormones (auxin and cytokinin) had significant effects on plant regeneration. Their influence was evident already on the early stages of cultivation. According to the results of the current study incubation of the mature seeds explants showed better response when cultured on MS medium supplemented with cytokinin BAP and auxin IAA. The seeds of both species became swollen quickly and germination occurred within the first two weeks of culture. Thus the duration from sowing of explant prior to its germination took a relatively short time span. The maximum seeds germination was observed on medium with 1 mg/L BAP plus 0.1 mg/L IAA for *J. warleyensis* and 0.5 mg/L BAP plus 0.1 mg/L IAA for *J. vicaria.*

The effectiveness of MS basal medium supplemented with BAP and IAA is demonstrated for better regenerants development. Thus, the growth regulators increase the regeneration ability of explants on 46% compared to control samples in both species (Fig.1).

Comparing effects of BAP+IAA proved to be more efficient than kinetin+IAA on regeneration in both species. The combination of kinetin in all concentrations with 0.1-1.0 mg/L IAA exerted negative effects on shoot proliferation in both species.




For *J. vicaria* the appearance of the first seedlings were observed in two weeks after sowing. For *J. warleyensis* the longer duration and a higher content of hormones were noticed. The seedlings were appeared in three weeks after sowing, true leaves have formed a week later. Thus, the species peculiarities influenced also on plantlets number to total number of sterile explants.

The experiment on MS medium supplemented with cytokinin exceeding auxin 5 times has shown the shoot proliferation activity in *J. vicaria*. One seed has given the regenerants with well-developed leaves passing callusing. Mean number of leaves per explant varied significantly depending on species. The maximum leaves formation in *J. vicaria* was achieved in the MS medium supplemented BAP (0.5mg/L) in combination with IAA (0.1mg/L). This ratio also showed more increase in all the parameters measured when compared to other combined concentrations (Table 1). Thus, BAP 0.5 mg/L + IAA 0.1 mg/L promoted a considerable increase in the number of leaves per explant in *J. warleyensis* and *J. vicaria* relative to that induced by combinations of BAP+IAA in ratio 1:1 (0.1 mg/L and 0.1 mg/L respectively).

**Table 1. Growth performance *in vitro* plantlets derived on basal media with different growth ratio regulators after four weeks of cultivation**

| **Treatment** | ***J. vicaria*** | | ***J. warleyensis*** | |
|---|---|---|---|---|
| | Plantlets height (mm) | Number of leaves | Plantlets height (mm) | Number of leaves |
| control | 19±0.7 | 2.6±0.13 | 17±0.6 | 2.2±0.15 |
| BAP | | | | |
| 0.1 | 41 ±2.9 | 4.1±0.33 | 42±2.1 | 3.7±0.2 |
| 0.5 | 76±3.5 | 7.8±0.34 | 53±2.8 | 4.5±0.3 |
| 1.0 | 58±2.2 | 5.5±0.24 | 84±4.2 | 5.3±0.7 |
| BAP+IAA | | | | |
| 0.1/0.1 | 62±3.2 | 3.9±0.17 | 47±2.5 | 3.7±0.2 |
| 0.5/0.1 | 83±4.3 | 7.2±0.34 | 74±3.8 | 6.5±0.35 |
| 1.0/0.1 | 72±3.1 | 6.5±0.3 | 81±3.5 | 6.3±0.4 |

One seed develops regenerants with several adventitious shoots. *J. warleyensis* required greater concentration of cytokinin for induction of shootings compared to *J. vicaria* (1.0mg / L and 0.5mg/l relatively). Thus, cytokinin is the basic factor broken the apical dominance and stimulated the proliferation of adventitious shootings.It was however noted that the both junos species resulted the plantlets inhibition, subsequently not viable regenerants and reduced plant heights in media series produced plantlets when the BAP and IAA concentration was increased from1.0 to 2.0 mg/L.The regenerants were unhealthy, and eventually died after week of cultivation

The MS media containing 0.5mg/L BAP + 0.1mg/L IAA was optimal for production of higher plantlets compared to other combinations.

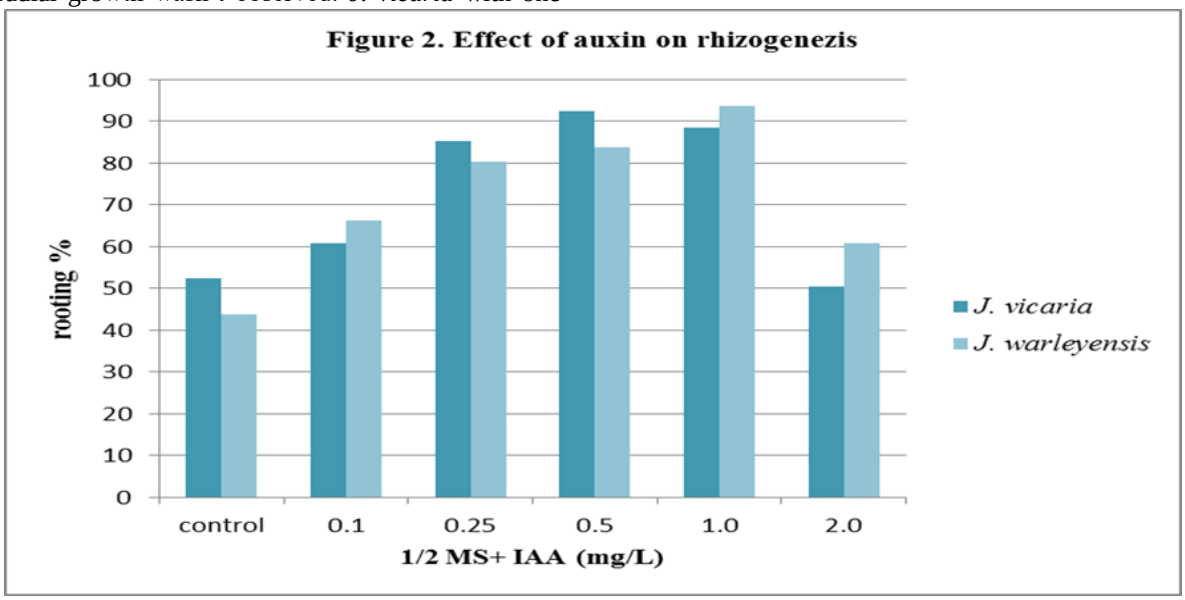
The experience with explants tested in plantlets formation on other culture media (combination kinetin+ IAA) did not show any response in regenerants initiation.

IAA concentration at 1.0- 2.0 mg/l range inhibited the aerial system development. The formation of true leaves was not observed even after the third passage. IAAexceeding 2.0 mg/l causes the shoots death. Thus, the optimal concentrations of BAP for seeds germination and shoot

induction was 1.0 mg /L for *J. warleyensis*, and 0.5 mg/L for *J. vicaria.* The shoots elongation takes no more than 2 weeks for both species.

The effect of auxin IAA in 0.1-2.0 mg /L concentration at rooting induction was studied (Fig.2). Rooting in primary explant was observed in 2 weeks after maintaining a sterile culture medium with auxin for both species. Increasing IAA concentration from 0.1 to 0.5 mg /L in the medium inhibits completely the aerial growth and induces the rooting. But intense radial growth wasn't observed. *J. vicaria* with one real leaf has shown spontaneous rooting. At that, the root formation for both species, largely stimulates by IAA in 0.5 mg/L concentration and separately from cytokinin.*J. vicaria* root induction with IAA at concentrations of 0.5- 1.0 mg /L resulted in 92 and 88% rooting respectively. For *J. warleyensis* 83% and 93% rooting was achieved with 0.5-1.0 mg /L IAA.The lowest rooting frequency was recorded on MS medium devoid of auxins in both species (52% and 43%).

**Figure 2. Effect of auxin on rhizogenezis**




The root growth *in vitro* depends on concentration of agar in nutrient medium also. Rooting of shoots in a dense medium is difficult. Thus, the decreasing of agar concentration from 0.6% to 0.5% significantly increased the rooting.

MS medium is enough to support the maximum growth of the plant. It's noticed, that the concentration of macro- and microelements in this medium is the reason of prolific plantlets growth incubated on this medium. Thus, better plant growth was observed on MS medium compared to 1/2MS.However, the tendency of higher survival rates in rhizogenesis stage was observed on MS medium with half mineral salts concentration.

Thus, the stimulating effect of cytokinin 6-BAP was manifested in the narrow range of concentrations (0.5 and 1.0 mg/L). The effectiveness of stepwise obtain plantlets by sequential converting of explant culture conditions was observed.

## IV. CONCLUSION

1) The scheme of introduction in culture of *Juno* species is developed.

2) The morphogenic effect of cytokinin is implemented more actively in 10: 1 ratio (BAP 1.0mg/l+ IAA 0.1 mg/l) for*J. warleyensis*, and less level 5:1 (BAP 0.5mg/l+ IAA 0.1 mg/l) for *J. vicaria*.It can be explained by that *J. vicaria* has a wider distribution area, hence a wider adaptive capabilities then *J. warleyensis.*

3) It is revealed that the presence of auxin in an amount no more than 0.5 mg/l effects on rhizogenesis positively.

The seed germination *in vitro* has shown the formation of cotyledonary leaf in just three weeks, then the first and the second true leaves.Thus the period from seedling to immature stage takes no more one month under laboratory conditions, whereas in open ground this period takes two or three years.

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