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Formulation, characterization and impact of an ionically crosslinked hydrogel HPMC and Xanthan gum on growth traits of soybean (*Glycine max* L.) under irrigation levels

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Abstract

Create and characterize novel hydroxy propyl methyl cellulose (HPMC) -Xanthan gum ionically cross-linked hydrogel was the aim of the current investigation. Different xanthan gum and cross-linker ratios were used at a specific HPMC ratio to create the hydrogel. FTIR, Thermal analysis (TGA& DSC), SEM, and swelling degree index were used to assess the hydrogel that was formed. One of the factors utilized to decide that Formula HPXa 5 was the best was its high water uptake. In order to study the effects of HPXa 5 application on soybean plants cultivated under two irrigation regimes (D1, every 7 days, or D2, every 10 days), HPXa 5 was applied at ratios of 0, 2, 3, and 4 g/kg soil.

The findings showed that plants which received irrigation every seven days had higher growth parameters, photosynthetic pigments, and lower levels of osmolytes (proline), non-enzymatic antioxidants (phenolic and flavonoid compounds), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and antioxidant enzymes (catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) and glutathione reductase (GR)) than plants that received irrigation every ten days. Regarding the HPMC-Xanthan gum hydrogel effect, it was clear that hydrogel application at all concentrations significantly increased the majority of the measured parameters (growth parameters, photosynthetic pigments, content of proline, phenolic and flavonoid compounds, activity of CAT, POX, SOD, accompanied by significant decreases in MDA, and H_2O_2 content) in comparison to corresponding controls in soybean plants were irrigated every seven or ten days. It is clear that the root's dry weight responds to HPMC-Xanthan gum more strongly than the responds Increases in total chlorophyll and decreases in MDA and H_2O_2 owing to 2g/kg HPMC-Xanthan gum were more pronounced in plants that received irrigation every 10 days than in plants that received irrigation every 7 days. It is important to note that HPMC-Xanthan gum hydrogel can be utilized as a useful tool to lower the amount of water needed for irrigation of soybean plants.

Keywords: Soybean, HPMC, Xanthan Gum, ionically cross linked Hydrogel, Irrigation levels, Antioxidant system, Phosphated cross linked

1. Introduction

By 2030, there will be a severe water crisis due to increase in Global water consumption. In most parts of the world today, the agricultural sector uses more than 70% of the fresh water, which results in water scarcity [1,2]. At the same time, evaporation and runoff cause an average loss of 63% of the water delivered to agricultural areas [3]. Water stress induced several devastating effects on plants via disturbing various physiological and biochemical processes as carbon assimilation rate, leaf gas exchange, and photosynthesis thus reduced the plant growth [4]. In addition, excessive reactive oxygen species (ROS) produced by water stress led to oxidative stress, which is harmful to proteins, lipids, carbohydrates, photosynthetic pigments, and nucleic acids [5].

Therefore, plants developed an antioxidant defence system (both enzymatic and non-enzymatic) detoxifying and balancing excess ROS. Antioxidants that are enzyme-based help to maintain defence against oxidative stress. The main antioxidant enzyme, superoxide dismutase (SOD), is responsible for converting ROS into H_2O_2, which is then buffered by the enzymes peroxidase (POD) and catalase (CAT) [6]. Carotenoids, ascorbic acid, and phenolic substances are recognized as the three most important non-enzymatic antioxidants [7]. Additionally, plants increase the production of osmolytes like glycine betaine, proline, free amino acids, etc. to reduce the negative impacts of water stress [8].

Therefore, it is necessary to develop new techniques and technologies to increase water holding capacity of soil and increase crop resistance to water stress. One of these techniques is using hydrogel soil additives [9].

Cross-linked hydrophilic polymer structures are known as hydrogels (super absorbent polymers).

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When hydrogels come into touch with water, they transform into gels and can absorb 400 to 1600 times more water than they did before [10, 11]. The molecular weight, formation, and structure of hydrogel affect how much water is absorbed [12]. In addition, Ideal hydrogel materials must be characterized as non-toxic material, have high-water absorption capacity, a low cost-effective material, high durability and stability, photo stable material, pH become neutral after swelling in the water, and biodegradable without formation of toxic species [13-15]. Numerous studies stated that hydrogels can enhance soil physical properties, chemical and biological properties especially in arid and semi-arid areas (porosity, bulk density, water holding capacity, soil permeability, infiltration rate) [11, 12, 16]. Because of the decrease in soil compaction due to increase in soil porosity, seed germination and seedling emergence rates, root growth and density, and soil erosion were all improved [17]. It was noted that adding hydrogels to sandy soil improved water availability to plants by decreasing drainage loss, swelling retention pores, and reducing soil hydraulic conductivity [18]. According to Bakass et al. [19] , the water holding capacity of sandy soil was enhanced from 171% to 402% by adding hydrogel at 2 g/kg to soil. Hydrogels may affect nutrient-use efficiency in addition to the prolonged release of water by retaining the nutrients in the swelled mass and lowering their losses [20-23]. In general, super absorbent polymers increased soil water holding capacity, decreased irrigation frequency, decreased wasting of water and nutrition materials of soil, increased soil aeration, and caused better plant growth and enlargement and increased yield under water stress conditions [24-30]. Likewise, application of hydrogel in drought-affected soil increased the plant survival time owing to water saving in rhizosphere and sufficient soil moisture [31] and improved germination, plant growth, and nutrient uptake[32,33]. Recently, Prisa and Guerrini [9] showed that addition of hydrogel improved growth and quality of Zea mays and Solanum lycopersicum. The increase in average germination time and plant development increased in direct proportion to the quantity of hydrogel capsules sown into the soil.

It is important to note that hydrogels contain cellulose, protein and starch in their structures [34-36]. In this work, HPMC-Xanthan gum was used as the hydrogel. Xanthan gum is a polysaccharide used as food additive, thickening agent, emulsifier and stabilizer that prevent ingredient from separating EFSA Panel on Food Additives and Nutrient Sources [37]. The fermentation of glucose and sucrose results in the production of xanthan gum. It is made up of pent saccharide repeating units with the molar ratios 2:2:1 of glucose, mannose, and glucuronic acid [38].

The most significant leguminous seed crop used for human consumption, industry, and animal feed is soybean (*Glycine max* L.), which is planted in almost every country in the world. It has good nutritional properties, including high protein content (40–42%), oil content (18–22%), macronutrients, and mineral content [39]. Water stress can affect soybeans [40]. It requires large quantities of water for a high yield [41], therefore, water stress has a serious effect on soybean production. Even a short-term of drought during the growing season can reduce yields by 30% to 80% [42].

The aim of the current investigation was to create and characterize novel hydroxy propyl methyl cellulose (HPMC)-Xanthan gum ionically cross linked hydrogel for usage as soil amendments. Also Its effect on growth parameters and biochemical changes in soybean leaves in response to two irrigation levels were examined.

2. Materials and Methods

The hydroxy propyl methyl cellulose (HPMC) was imported from Dow CELLOSIZE™ Texture K100 M HPMC. Yields viscosity of 100,000 cP at low addition levels. Xanthan was purchased from Sigma. Sodium tri-metaphosphate (STMP) and sodium sulfate were utilized as cross linkers.

Hydrogel (HPMC-Xanthan gum) Synthesis

Ten grams of HPMC were dissolved in 250 ml of water and then various amounts of xanthan gum (2.5.7.10) g were added gradually while the mixture was vigorously mixed. After vigorously agitating the mixture for one hour, it was then submerged in a pot of boiling water. The liquid was added 10 ml of 1 N NaOH after being chilled to 55 ^{o}C. After that, the cross-linking process was started by adding various of cross linker concentrations (Table 1) and 20% sodium sulphate (based on the weight of dry xanthan). By bringing the pH to 7 with 1 N HCl after 5 hours, the process was put to an end. Following that, the modified HPMC hydrocolloid polymer was separated using centrifugation (4000 rpm for 20 min), rinsed with water (150 ml), and dried in an air oven at 55 ^{o}C to prepare powder [43].

Hydrogel characterization

Fourier transformed infrared spectroscopy (FT-IR)

HPMC Xanthan gum and a specific hydrogel sample were subjected to ATR-FTIR measurements using a Bruker VERTEX 80 (Germany) Platinum Diamond ATR, which uses a diamond disc as an internal reflector and measures refractive index with a resolution of 4 cm1.

Thermo gravimetric analysis (TGA)

Using a Shimadzu TGA-50 thermo gravimetric analyzer, Columbia, EUA, in a nitrogen atmosphere, and heating the sample at a rate of 10 oC/min between ambient temperature and 600 oC, a thermal gravimetric analysis (TGA) analysis was carried out on a chosen hydrogel sample.

Table (1): Composition of different formulation of different HPMC-xanthan hydrogel

Hydrogel code	HPMC(g)	Xanthan (g)	STAMP Cross linker (g)
HPXa1	10	2	0.02
HPXa2	10	5	0.05
HPXa3	10	7	0.07
HPXa4	10	10	0.1

Differential scaning calorimetry (DSC)

A Shimadzu DSC-60 differential scanning calorimeter (DTA), manufactured in Columbia, EUA, was used to test a hydrogel sample. Similar to TGA, there was a similar scan rate and heating temperature range.

Scanning electron microscopy (SEM)

Scanning electron microscopy, FESEM QUANTA 250, was utilized to examine the hydrogel sample's surface morphology and cross-section topography. To reduce the effect of charging during the examination, gold was splattered on the dried and wet samples.

Swelling degree (SD)

According to Benhalima et al. [44], measurements of swelling degree (SD) were made in demineralized water at a temperature of 25 °C. Equation (3) was used to calculate the swelling degree under the examined swelling media at time t and expressed in g/g of dry hydrogel:

$$SD = \frac{W_t - W_d}{W_d}$$

Where Wd is the weight of the samples before immersion in swelling media (i.e. dry weight), and Wt is the weight of the sample at each time.

The chosen dependent variable was the swelling degree (SD). The swelling capability was measured by carefully weighing the samples before and after immersion in demineralized water for 24 h (Y=SD24h)

Pot experiment

To investigate the impact of HPMC-Xanthan gum hydrogel on growth parameters, chlorophyll content, and biochemical changes in soybean leaves in response to irrigation levels, a pot experiment was conducted at the Botany Department's greenhouse at the National Research Centre, Dokki, Cairo, Egypt, through two summer seasons.

Plant material

Seeds of soybean (*Glycine max* L., *CV* Giza 111) were supplied by Agricultural Research Centre (ARC), Agriculture Ministry, Giza, Egypt.

Design of the experiment

This experiment was set up as a two-factor, completely randomized block design (RCBD), with six replications. The first factor consisted of four levels of HPMC-Xanthan gum hydrogel (HPXa 5), which was chosen as the best preparation due to its high swelling index and high water uptake (0, 2, 3 and 4 g/kg soil), and the second factor consisted of two irrigation levels (D1, once/7 days and D2, once/10 days). 11 kg of soil (air dry base) were put into the pots, which are 35 cm^3 in size. The mechanical and chemical analyses of the sandy soil are shown in Table 2. The soil was then mixed with HPMC-Xanthan gum hydrogel. After being sterilized with sodium hypochlorite, soybean seeds were sown in pots. For each irrigation level, there is a control group, a group of plants grown without hydrogel supplementation, and a group of plants grown with hydrogel supplementation at the following concentrations: (i) 2 g/kg soil; (ii) 3 g/kg soil; and (iii) 4 g/kg soil. Following the complete establishment of seedlings and the selection of the strongest seedling in each pot (21 days after sowing), watering treatments were initiated. The fertilization was carried out in accordance with the agricultural ministry's advice.

Soybean growth

For determining morphological traits, such as root and shoot length (cm), number of leaves per plant, root and shoot fresh weight (FW) (g), and root and shoot dry weight (DW) (g), plant samples were collected after 60 days after planting. After drying a plant sample in an oven for 48 hours at 50°C, dry weight was determined.

Biochemical measurements

Photosynthetic pigments i.e. chlorophyll a, chlorophyll b and carotenoids were estimated in fresh leaf tissues using the method of Lichtenthaler and Buschmann [45]. Proline content was extracted and calculated according to Bates et al. [46]. Total phenolic compounds (TPC) were determined using a Folin-Ciocalteau colorimetric assay modified by Elzaawely and Tawata [47]. Total flavonoid content (TFC) was measured by using the aluminum chloride technique modified by Chang et al. [48]. The malondialdehyde (MDA) content was evaluated using the method of Heath and Packer [49]. Hydrogen peroxide (H_2O_2) was determined following the method of Yu et al. [50]. Enzyme extracts were prepared according to the method of Chen and Wang [51]. Catalase (CAT) activity was determined by following the decrease in absorbance at 240 nm according to Chen and Wang [51]. Superoxide dismutase (SOD) activity was evaluated by nitroblue-tetrazolium reduction method [51]. Peroxidase (POX) activity was estimated by Kumar and Khan [52]. Glutathione reductase (GR) activity was estimated according to Rao et al. [53] by following the increase in absorbance at 340 nm.

DNA isolation and PCR

Total genomic DNA was isolated from young and fresh leaves of treated soybean plants according to modified cetyl tri methyl ammonium bromide CTAB method described by Khaled and Esh [54]. Ten ISSR primers were screened for the production of polymorphic products from all treated plants under study. Only five primers showed polymorphic patterns and were selected, as shown in Table 3. Moreover, polymerase chain reaction (PCR) was carried out within 15 µl reaction volumes. containing 1 µl plant genomic DNA, 7.5 µL Master Mix (Gene Direx one PCRTM), 1 µL template DNA and 1 µL primer.

PCR was programmed as: an initial denaturation at 94°C for 3 min, 45 cycles each of 94°C for 1 min, then 55ºC for 30 sec., 72ºC for 40 sec. for annealing and final extension at 72ºC for 10 min. Amplification products were electrophoresed on 1.5 % agarose in 1×TAE buffer. Then gel was stained with ethidium bromide and documented using gel documentation system.

Table (2): Physiological and chemical analysis of soil used in pots

Characteristics	Value
Physical properties	
Particle size distribution	
Coarse Sand%	73.8
Fine Sand%	15.5
Silt%	6.5
Clay%	4.2
Texture Soil	Sandy
Chemical properties	
Organic matter content%	1.24
pH	7.8
EC ds/m	0.74
Cations meq/L	
Na^+	4.15
K^+	0.23
Ca^{++}	1.84
Mg^{++}	1.25
Anions meq/L	
HCO_3	0.64
CO_3	Nil
SO_4	0.93
Cl^-	5.6

Statistical analysis

Analysis of variance was used to statistically analyze the average of two seasons' supply of data. According to Silva and Azevedo [55], the differences between means were evaluated by the least significant differences (LSD).

Table (3): Names and sequences of selected ISSR primers utilized in this study.

Primers	Primer Code	Primer Sequence
IS-01	844 B	(CT)8 GC
IS-02	17898A	(CA)6 AC
IS-03	HB 10	(GA)6 CC
IS-04	HB 11	(GT)6 CC
IS-05	HB 12	(CAC)3 GC

3. Results

Characterization of hydrogels

Various hydrophilic natural polymers were used in the effort to plan and represent the HPXa hydrogel matrix and its use in drought stress. HPXa hydrogel-based matrix has not yet been researched for applications including drought. The planned application could be used on an industrial scale and was affordable. Following analysis of the factor design, the optimal formula was selected as the most promising soil substrate conditioning materials. A thorough analysis of the selected materials was carried out to demonstrate their potential use in order to regulate soil moisture and enhance plant growth under drought stress.

FT-IR spectroscopy

Illustrative spectra of nominated polymers (HPMC, xanthan gum) and phosphate cross linked of optimized hydrogel HPXa were exposed in Figure 1. At 3437 cm^{-1}, HPMC showed a clear broadband zone related to O-H stretching vibrations. C-H and C-O bonds were allocated bands at 2940 and 1060 cm^{-1} [56]. A significant stretching vibration at 1655 and 1119 cm^{-1} attributed to CH3 and C-O-C bonds was also demonstrated by HPMC [57]. Due to axial O-H deformation, xanthan gum produces stretching vibrations at 3413 cm^{-1}. The stretching vibrations of the C-H group cause the peak at 2880 cm^{-1}, while the stretching vibrations of the C-O group cause the peak at 1621 cm^{-1}, and the bands near 1407 cm^{-1} are caused by the axial deformation of the C-O component of the enol [58]. The hydrogel's FT-IR spectra revealed that STMP interacted with HPMC and xanthan gum.

A decrease in hydroxyl groups and no change in the quantity of carbonyl groups are anticipated because hydroxyl groups from xanthan and HPMC are implicated in the reaction with STMP and are transformed to O-P linkages in the crosslinked product. The absorbance at the maximum of the peaks caused by hydroxyl groups (3410 cm-1 to 3470 cm^{-1}) and carbonyl groups (1644 cm^{-1} to 1660 cm^{-1})

was taken from the FT-IR spectra to evaluate this behaviour. (Figure 1 a & b). The STMP polar molecules and the HPMC and Xanthan gum chains interacted with one another, shifting the 1296 and 997 cm^{-1} (P=O and P-O-P bands, respectively 8, 9) to lower energy levels on the products. The presence of a peak at 1015 cm^{-1}, which is connected to the production of P-O-C bonds between the cross linker and the polysaccharide, indicating the cross linking of the product. Cross-linked HPXa prepared hydrogel showed evidence of interaction between HPMC and Xanthan polymers with STMP cross linker (Figure 1C).

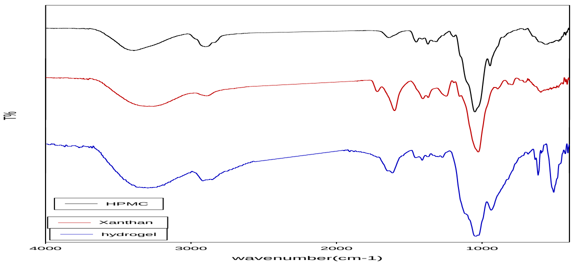

Fig.1. FT-IR spectra of (a) HPMC (b) xanthan gum (c) HPXa Phosphated cross linked hydrogel

Thermo gravimetric analysis (TGA)

A TGA was carried out to examine the hydrogel beads' heat stability and rate of deterioration. Figure 2 displays a progressive and sequential weight loss pattern for the chosen sample HPXa 5 TGA. The initial heat event causes water to evaporate, which results in a 2% weight loss. The temperature range of 30 to 170 °C was used to measure this weight loss. The subsequent weight loss of 5-10% in the range of 270-380°c is due to bond breakdown. About 80% of the sample was still present at this phase, which is equivalent to intermediate carbonaceous char material. Between 370 and 510°c, there is a 9% weight loss that is attributed to the oxidation of intermediate carbonaceous char components that have already formed [59].

TGA offers an effective way to investigate the produced hydrogel's thermal stability. For the optimization of process parameters, understanding degradation and the manner of decomposition under the effect of heat is highly advised. The TGA curve advances one step at a time. The range for decomposition was 270–380 °C. The hydrogel residue was 85% at the highest temperature. Figure 2 and Table 4 show that the sample's weight declined steadily as the temperature rose [60]

The primary criteria utilized to determine the thermal stability of the prepared hydrogel are TGA data related to the temperatures corresponding to weight losses of 15% (T15), 50% (T50), 90% (T90), and maximum (Tmax). The higher T15, T50, T90, and Tmax numbers are, the higher will be the thermal stability of the prepared hydrogel [61].

Table (4): TGA decomposition temperature of hydrogel

% weight loss at various temperature °C				**Decomposition temperature range °C**	**T_{15}**	**T_{50}**	**T_{90}**	**T_{max}**
200	300	400	Max					
98	95	89	85.5	270 - 380	More than 600	More than 600	More than 600	More than 600

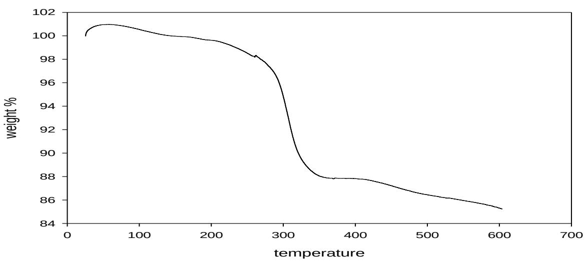

Fig.2. TGA thermo gram of HPXa 5

Differential scaning calorimetry (DSC)

The use of DSC allowed for the direct determination of whether a phase change is endothermic or exothermic as well as the reversibility of phase transitions, the temperature history, and the physical characteristics of the analysed materials. It also looked into possible interactions between various components of the hydrogel that had been synthesised. At 82.11 °C (45.01-110.2 °C), the hydrogel's melting transition was observed as a broad peak, which corresponds to the moisture loss (Figure 3). According to Figure 3 and Table 5, there was thermo behaviour variability in the temperature range of 270 to 380° C. The breakdown of cross linkers that were created in the hydrogel during preparation was the cause of the variability.

Table (5): DSC decomposition temperature °C

	Decomposition temperature °C			
	Initiation Temperature	Peak Temperature	Final Temperature	Tg
HPXa 5	270	300	380	37

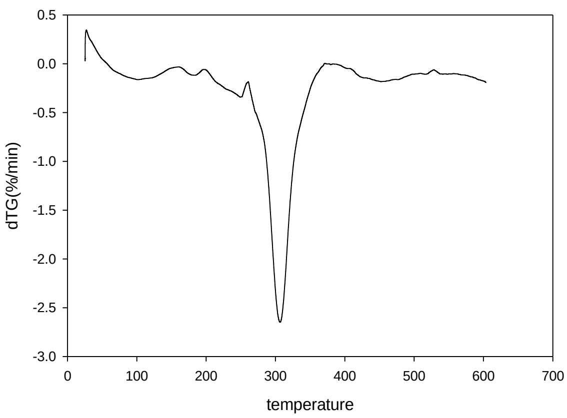

Fig.3. DSC thermo gram of HPXa 5

Scanning Electron microscopy (SEM)

To determine the surface topography of the HPXa hydrogel matrix, SEM examination was completed. Figure 4 shows examples of SEM images of HPXa hydrogel cross sections before drying and after swelling for 24 hours in demineralized water. SEM images displayed many channels and spherical pores with various pore sizes that connected polymeric networks. The ability of the matrix to create both macro-porous structures and micro cavities. This may lead to the formation of a porous or permeable gel layer that allows the release medium to slowly diffuse water out of the matrix by entering the water's matrix and moving towards the plant. Therefore, the presence of pores and a gelling structure on the HPXa hydrogel points to the involvement of both diffusion and erosion mechanisms in regulating the release of water from the optimized HPXa hydrogel matrix, which will affect and control soil substrate moisture and have an impact on plants under drought stress.

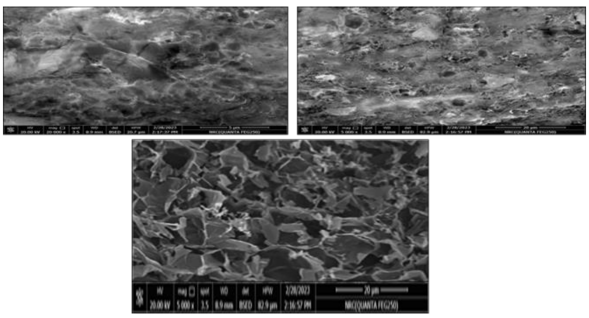

Fig.4. SEM cross section of selected hydrogel before at magnification 10,000 and 20,000 (upper two pictures) and after swelling for 24 hr. at magnification 5,000 in demineralized water (bottom picture).

Swelling degree index

The swelling behaviour of the hydrogel are presented in Table 6 and Figure 5 hydrogel which had higher xanthan gum content displayed improved swelling properties at a constant pH 7. Due to the presence of ions in the cross linker, the HPXa 5 formula's high xanthan and phosphate cross linker ratio demonstrated improved swelling capability. The gum's hydrophilicity increased as a result of the cross-linker's ion, which also increased the gum's swelling capabilities [62]. Therefore, the formula HPXa 5 was chosen as the best one based on the criteria of using a high water uptake hydrogel in a drought-stress application. Water diffusion into the hydrogel 3D network and the ensuing relaxing of the polymer chains are essential components of the water sorption mechanism. A swelling hydrogel's water transport phenomenon is greatly impacted by various factors including the chemical composition of hydrogel, its equlibrium water content and swelling rate among others [63].

Vegetative growth parameters

Table 7 shows that soybean plants irrigated every 7 days was characterized by higher growth parameters i.e. root and shoot length, number of leaves/plant, root and shoot fresh weight, root and shoot dry weight than those irrigated every 10 days.

Table (6): Swelling degree and water uptake

Hydrogel code	Swelling degree index %				Max Water uptake(ml/gm hydrogel)
	2hr	8hr	16hr	24hr	
HPXa1	100	120	150	180	180
HPXa2	100	210	280	300	300
HPXa3	100	250	290	350	380
HPXa4	100	260	300	360	390
HPXa5	100	300	400	480	500

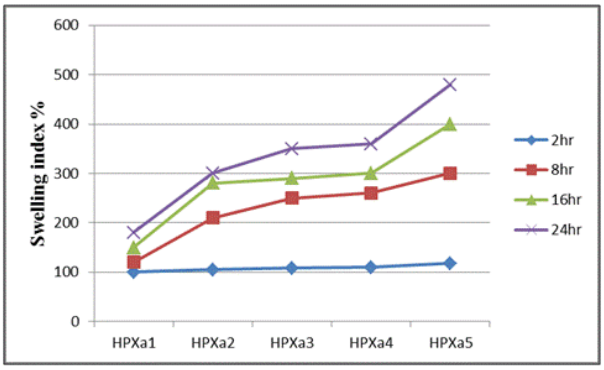

Fig.5. Swelling index of the HPXa hydrogel at pH **7.**

Hydrogel (HPMC-Xanthan gum) at all concentrations significantly increased most of growth parameters in plant irrigated either every 7 days or 10 days relative to corresponding controls. It was obvious that 2 g/kg soil of HPMC-Xanthan gum was the most pronounced treatments. Since, it significantly increased root dry weight by 96.43% in plant irrigated every 7 days and by 104% in plant irrigated every 10 days relative to corresponding controls. Likewise, it significantly increased shoot dry weight by 41.42 % in plant irrigated every 7 days and by 33.33 % in plant irrigated every 10 days relative to corresponding controls. It is obvious that response of root dry weight to HPMC-Xanthan gum is more pronounced than response of shoot dry weight.

Photosynthetic pigments

Table 8 shows that soybean plant irrigated every 7 days was characterized by significant increases in total chlorophyll (a+ b), and carotenoids than those irrigated every 10 days.

Hydrogel (HPMC-Xanthan gum) at all concentrations significantly increased chlorophyll a, chlorophyll b, carotenoids in plant irrigated either every 7 days or 10 days relative to corresponding controls. The increase in photosynthetic pigments was in opposite direction to hydrogel concentrations. It was obvious that 2 g/kg soil of HPMC-Xanthan gum was the most pronounced treatments. Since it increased total chlorophyll by 23.87% in plant irrigated every 7 days and by 56.49 % in plant irrigated every 10 days relative to corresponding controls. It is obvious that the percentage of increment of total chlorophyll to 2 g/kg soil of HPMC-Xanthan gum was higher in plant irrigated every 10 days than those irrigated every 7 days.

Table (7): Effect of hydrogel (HPMC-Xanthan gum) on some growth parameters of soybean plants grown under two irrigation levels (D1) once/7days and (D2) once/10 days

Treatments		Root length (cm)	Shoot length (cm)	Number of leaves/plant	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Irrigation levels	Hydrogel levels (g/kg soil)							
Irrigation levels								
D1		15.40	39.23	7.80	2.63	0.92	18.42	5.02
D2		13.63	35.79	7.63	2.58	0.75	17.28	4.56
LSD at 5% level		1.05	0.94	0.15	0.23	0.10	0.20	0.07
Hydrogel levels								
0		12.46	32.61	6.61	2.30	0.51	13.59	3.98
2		15.33	41.92	8.37	2.89	1.01	21.65	5.46
3		15.17	39.42	8.25	2.77	0.95	19.11	5.07
4		15.08	36.09	7.63	2.46	0.87	17.06	4.65
LSD at 5% level		1.49	1.34	0.21	0.33	0.14	0.29	0.09
Interaction between irrigation levels and hydrogel levels								
D1	0	13.25	34.25	7.21	2.33	0.56	14.45	4.08
	2	16.33	44.34	8.33	2.88	1.10	22.15	5.77
	3	16.00	41.33	8.33	2.86	1.04	19.39	5.29
	4	16.00	37.00	7.33	2.47	0.99	17.68	4.94
D2	0	11.67	31.00	6.00	2.28	0.45	12.73	3.87
	2	14.33	39.50	8.42	2.90	0.92	21.15	5.16
	3	14.33	37.50	8.17	2.68	0.86	18.82	4.85
	4	14.17	35.17	7.92	2.46	0.75	16.43	4.36
LSD at 5% level		2.10	1.89	0.30	0.47	0.20	0.41	0.140

Biochemical constituents of soybean leaf

Figure 6 show that soybean plant irrigated every 10 days was characterized by significant increases in proline content, MDA and H_2O_2 and non-significant increase in non-enzymatic antioxidant (total phenolic compounds and flavonoid content) than those irrigated every 7 days.

At all concentrations HPMC-Xanthan gum hydrogel increased proline content, phenolic compounds and flavonoid content accompanied by decreases in MDA, and H_2O_2 in plant irrigated either every 7 days or 10 days relative to corresponding controls. The increase in proline content, phenolic compounds and flavonoid content and decrease in MDA and H_2O_2 was in opposite direction to hydrogel concentrations. It was obvious that 2 g/kg soil of HPMC-Xanthan gum was the most pronounced treatment. Since it significantly decreased MDA and H_2O_2 by 15.09 and 6.63% in plant irrigated every 7 days and by 20.10% and 15.96% in plant irrigated every 10 days relative to corresponding controls. It is obvious that the percentage of decease of MDA and H_2O_2 due to 2 g/kg soil HPMC-Xanthan gum was higher in plant irrigated every 10 days than those irrigated every 7 days.

Enzymatic antioxidant

Figure 7 shows that soybean plant irrigated every 10 days were characterized by significant increases in antioxidant enzymes (CAT, POX, SOD, GR) than those irrigated every 7 days.

Table (8): Effect of hydrogel (HPMC-Xanthan gum) on photosynthetic pigments of soybean plants grown under two irrigation levels (D1) once/7days and (D2) once/10 days

Treatments		Chl. a	Chl. b	Chl. (a+b)	Carotenoid
Irrigation levels	Hydrogel levels (g/kg soil)	(mg/g fresh leaf tissues)			
Irrigation levels					
D1		3.337	0.783	4.118	4.499
D2		3.032	0.824	3.856	3.931
LSD at 5% level		0.004	0.004	0.005	0.003
Hydrogel levels					
0		2.567	0.610	3.178	3.602
2		3.479	0.912	4.391	4.567
3		3.410	0.865	4.275	4.418
4		3.280	0.827	4.106	4.273
LSD at 5% level		0.006	0.006	0.007	0.0041
Interaction between irrigation levels and hydrogel levels					
D1	0	2.877	0.701	3.569	3.854
	2	3.559	0.862	4.421	4.755
	3	3.487	0.802	4.289	4.711
	4	3.428	0.766	4.194	4.678
D2	0	2.267	0.519	2.786	3.351
	2	3.398	0.962	4.360	4.379
	3	3.334	0.927	4.261	4.124
	4	3.132	0.887	4.018	3.868
LSD at 5% level		0.009	0.009	0.010	0.006

At all concentrations HPMC-Xanthan gum hydrogel significantly increased CAT, POX, and SOD, GR in plant irrigated either every 7 days or 10 days relative to corresponding controls. The increase in CAT, POX, SOD, and GR was in opposite direction to hydrogel concentrations. It was obvious that 2 g/kg soil of HPMC-Xanthan gum was the most pronounced treatment. it significantly increased CAT, POX, SOD, GR by 16.94, 83.76, 53.62, and 19.70 % in plant irrigated every 7 days and by 12.62, 91.68, 37.18, 17.67 % in plant irrigated every 10 days relative to corresponding controls.

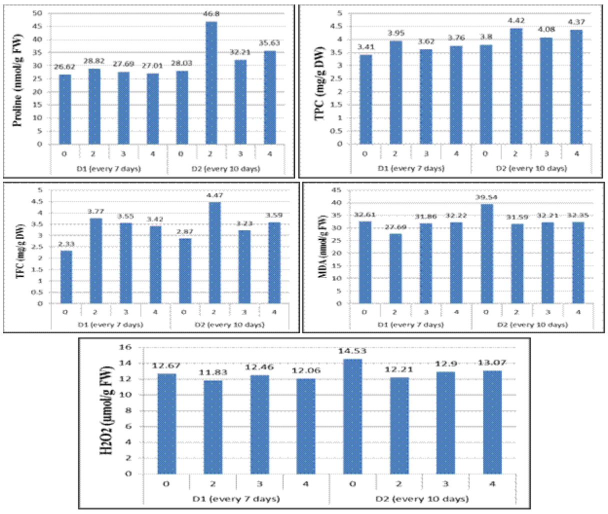

Fig.6. Effect of hydrogel (HPMC-Xanthan gum) on some biochemical composition of soybean plants grown under two irrigation levels (D1) once/7days and (D2) once/10 days

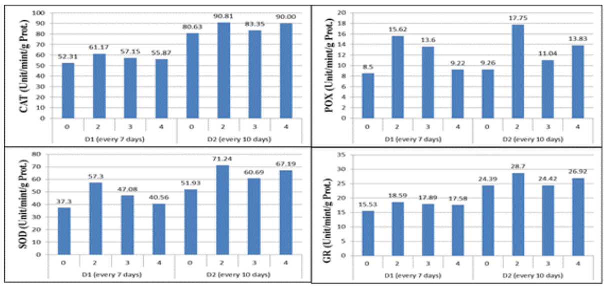

Fig.7. Effect of hydrogel (HPMC-Xanthan gum) on some antioxidant enzymes of soybean plants grown under two irrigation levels (D1) once/7days and (D2) once/10 days ISSR Molecular Markers

Table 9 and Figure 8 illustrated the effect of interacting hydrogel and drought stress on reproducible DNA fragments that detected by five

ISSR primers (Table 3). However, multiple fragments with different molecular weights were detected using these five ISSR primers, and the reproducible fragments distributed between monomorphic bands, polymorphic bands and unique bands. Moreover, there were 68 bands as total number of bands (TAF) with (85.92%) polymorphism percentage (PB %). It was noticed that the highest level of polymorphism was observed with IS-01 primer followed by IS-04 primer which showed (92.31 and 91.67%) polymorphism, respectively, while the lowest polymorphism was 80.00% with both primers IS-02 and IS-05. It was noticed that the detected bands were varied in number, polymorphism and range of its molecular weights between used ISSR primers (IS-02 and IS-05) amplified the highest number of bands (15 bands) with the lowest polymorphism percentage (PB %= 80%). With regard to IS-01, 13 bands were detected with this primer and molecular weights of these bands ranged between (1780.4 - 256.7bp), moreover, it distributed as 1 monomorphic bands (MB), 6 unique bands (UB) and 6 polymorphic bands (PB) with 92.31% polymorphism. Meanwhile, there were 15 bands with molecular weights (2169.8 - 347.8 bp) and 80.00% polymorphism were detected using IS-02 primer, and distributed as 3 (MB), 7 (UB) and 5 (PB). Moreover, there were 13 bands with molecular weights (2148.0 - 489.9 bp) and 84.62% polymorphism were detected using IS-03 primer, and distributed as 2 (MB), 4 (UB) and 7 (PB).

On the other hand, with IS-04 and IS-05, there were 12 and 15 amplified bands with molecular weights (2112.3 - 452.5 bp) and (1644.9 - 249.9 bp) were detected using IS-04 and IS-05, respectively. These previous bands were distributed as (1 MB, 6 UB and 5 PB) and (3 MB, 7 UB and 5 PB) with IS-04 and IS-05, respectively. Moreover, there were different polymorphic bands appeared under different treatments in this study (Fig.3). However, the distributions of these detected bands was varied under used treatments (hydrogel concentrations and irrigation level), such as, there were bands appeared as a result to water stress only and these were found only in control plants under all hydrogel concentrations and disappeared under water stress. On the other hand, there were polymorphic bands was observed as a result to the effect of hydrogel concentrations under two irrigation levels.

Table (9): Effect of hydrogel (HPMC-Xanthan gum) on molecular characteristics and polymorphism of soybean plants grown under two irrigation levels (D1) once/7days and (D2) once/10 days

Primers	Marker size (bp)	Amplified bands				PB%
		TAF	MB	UB	PB	
IS-01	1780.4 - 256.7	13	1	6	6	92.31
IS-02	2169.8 - 347.8	15	3	7	5	80.00
IS-03	2148.0 - 489.9	13	2	4	7	84.62
IS-04	2112.3 - 452.5	12	1	6	5	91.67
IS-05	1644.9 - 249.9	15	3	7	5	80.00
Total		68	10	30	28	85.92
Average		13.6	2.0	6.0	5.6	-

TAF = Total amplified fragments, MB= Monomorphic bands, UB= Unique bands, PB =Polymorphic bands and PB (%) = Percentage of polymorphism.PB (%) = UB + PB / TB

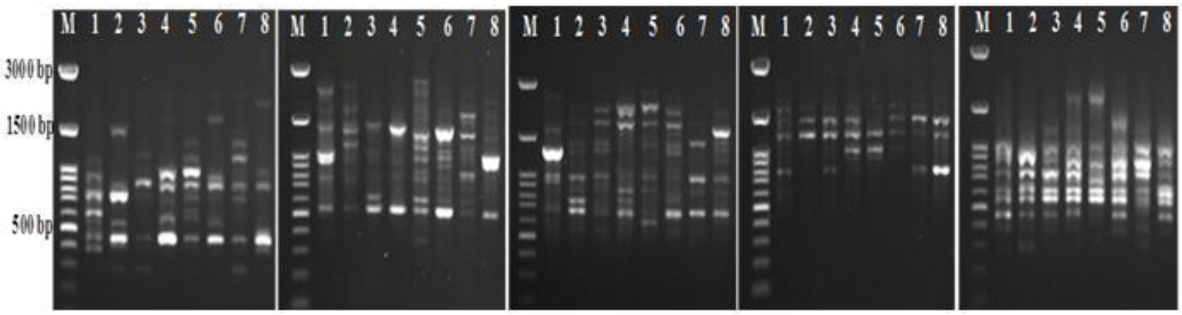

Fig.8. Effect of hydrogel (HPMC-Xanthan gum) on ISSR-based PCR fragments of soybean plants grown under two irrigation levels (D1) once/7days and (D2) once/10 days

4. Discussion

First of all, it must be mentioned that during summer season under normal conditions, soybean plants must be irrigated twice/week. This work includes two irrigation levels one/7 days and one/10 days. It was noted that soybean plants irrigated every 10 days was characterized by significant decreases of different growth parameters relative to those irrigated every 7 days (Table 7). These decreases could be the result of disorder brought on by drought stress on physiological and biochemical processes, such as the levels of plant growth regulators, photosynthetic assimilation activities, and activities of key enzymes involved in several essential metabolic processes [64]. The reductions in cell elongation, cell turgor, cell volume, and ultimately cell growth that occur in response to drought stress may be the cause of the losses in plant height [65]. Under drought stress, the observed decrease in shoot and root dry weights may be the result of reduced photosynthesis, decreased gas exchange activities, CO2 fixation, and ROS-induced cell damage [66,67]. Additionally, drought impacted the way plants interacted with water, lowered shoot water content, led to osmotic stress, inhibited cell expansion, cell division and consequently growth of plants as a whole[68].

It was noted that photosynthetic pigments of soybean plants irrigated every 10 days was characterized by significant decreases relative to those irrigated every 7 days (Table 8). These reductions in photosynthetic pigment contents might be caused by chloroplast lipids oxidation, disorganization of thylakoid membranes, damaging the photosynthetic apparatus, chlorophyll degradation by proteolytic enzymes such as chlorophyllase that is responsible for degrading chlorophyll and deterioration in chloroplast [69-73] Drought prevents or reduces photosynthetic carbon fixation generally through limiting the entry of CO_2 into the leaf or directly stopping its metabolism [74].

Another common plant response to drought stress is accumulation of osmo-protectants such as proline [75]. The main roles of these compounds are improving osmoregulation, protecting the structure of different bio-molecules, membranes and scavenging free radicals [76]. Proline accumulation in plants has been observed to be more than 70 times higher under water stress conditions than under unstressed conditions [77, 78]. Ashraf and Iram [64] mentioned that the increase level of proline in cowpea plant acts as an indicator of its high drought tolerance. Proline is also regarded as an antioxidant and helps to maintain redox equilibrium, which protects plant cells from drying out [79].

Plants have different protection mechanisms for reduction or elimination of ROS that are produced due to abiotic stress. According to Guo et al. [80] and Hosseini et al. [81], plants respond to adverse effects of water stress by improving the production of non-enzymatic defence systems as secondary metabolites (phenolic and flavonoid compounds) and antioxidant enzymes (SOD & CAT), which scavenge ROS[73, 82].

Regarding non-enzymatic defence systems, Rivero et al. [83] verified the increase of total phenolic compounds in response to abiotic stress. The increase of total phenolic content played a significant role in regulation of plant metabolic processes [84]. Due to disruptions in numerous metabolic processes in plant cells brought on by stress, the levels of phenolic contents may have increased during drought stress [85]. Through their reactivity as electron or hydrogen donors, to stabilize and delocalize the unpaired electron, and from their function as transition metal ions chelator, phenolic compounds are potent antioxidant scavengers of free radicals [86]. Additionally, phenolic substances serve as a substrate for numerous antioxidant enzymes, reducing the damage caused by water stress [87].

Plants are protected against environmental stress by secondary metabolites of phenolic origin called flavonoids [88]. Water stress dramatically boosted the flavonoid concentrations in maize plants, according to Ali et al. [89]. The increases of total amount of both phenolic and flavonoid compounds increased their antioxidant activity [90].

Drought stress causes an imbalance between the generation and detoxification of ROS, which leads to their accumulation [91]. Figure 6 demonstrates how drought stress caused soybean plants to significantly raise their H_2O_2 and MDA levels. Water stress led to the build-up of ROS, especially H_2O_2, which damaged plants via oxidation and created MDA [92]. MDA is a biomarker to estimate the level of lipid peroxidation or damage to plasma lemma and organelle membranes that are connected with damages triggered by ROS due to environmental stresses[93]. According to Hossain et al. [94], these increases may be caused by insufficient induction of the antioxidant system.

Antioxidant enzyme system is one of the protective mechanisms because of its ability to scavenge ROS. There is a strong association between tolerance to oxidative stresses and increase in concentration of antioxidant enzymes [95]. SOD, CAT, POX and GR are responsible for ROS scavenging [96]. Drought increased SOD, CAT, POX and GR activities in soybean leaves (Figure 7). Abdelgawad et al. [97] and Havrilyuk et al. [98] mentioned that these increases in enzyme activities owing to its resistance under water deficiency. SOD is the first defence enzyme that converts superoxide to H_2O_2, which can be scavenged by CAT and different classes of POX and GR. These enzymes are the most important antioxidant causing the breakdown of H_2O_2 to water and oxygen molecule [96].

It was noted from Tables 3 hydrogel application induced the growth parameters of soybean plant irrigated every 7 days or 10 days relative to corresponding controls. Moreover, incorporating different levels of HPMC-Xanthan gum hydrogel (2, 3 and 4 g/kg soil) as super absorbent polymers into the soil under irrigation level (once/7 days) significantly increased soybean plant growth parameters (Table 7), total photosynthetic pigment (Table 8), (osmolytes) proline, (non-enzymatic antioxidant) phenolic and flavonoid compounds (Figure 6), (antioxidant enzymes activity) catalase, peroxidase, superoxide dismutase, glutathione reductase (Figure 7), accompanied by significant decreases in MDA, and H_2O_2 (Figure 6) than those irrigated once/10 days.

These findings are in line with those stated by Yazdani et al. [20]; Galeș et al. [99] who showed that application of super absorbent polymer under drought stress increased the total dry weight and grain yield of soyabean by increasing water-holding capacity of soil , water use efficiency, and improving aeration of the soil. In addition, Khadem et al. [100] ; El-Asmar [101] stated that the application of hydrogel polymer to the soil enhanced seed germination, leaf water content, leaf chlorophyll content, root development, plant growth, minimized nutrient losses by leaching and decreased the adverse effects of water stress under arid region. Similarly under drought-stress conditions, Jarvis and Davies [102] described that super absorbent polymers improved leaf relative water content and photosynthesis rate that would enhance plant growth .

Moreover, Rezashateri et al. [103]) reported that hydrogels seemed to increase root growth. Since, the formation of a better root system is favourable to obtain efficient utilization of water and nutrients resources [104]. Liao et al. [105] found that incorporating super absorbent polymer into the soil not only increased root biomass and adjusts root distribution, but improved the amount of water absorbed through increasing root length density and root surface-area density at the upper soil layer under a dry land cropping system [106]. It is well known from several researches that hydrogel can utilize the existing water and nutrients in the soil, create a water pool in the plant root zone, and release them gradually through a diffusive mechanism when the soil becomes dry. In this manner, the plant root may regulate or effectively use the drainage or evaporation of watered water [107, 108]. Additionally, applying hydrogel as a soil conditioner has the potential to increase water use efficiency. Its use decreased the amount of water needed for the ideal crop by 38% to 40% [108, 109]. Tongo et al. [110] stated that the interaction effect between drought stress and super absorbent polymers on the different growth parameters, proportion of root dry weight to aerial dry weight, photosynthetic pigments, catalase and peroxidase enzyme activity was significant. Recently, Havrilyuk et al. [98] concluded that the use of superabsorbent increased plant growth parameters under the conditions of the water shortage due to increased water availability and nutrients which consequently increased the cell division, cell expansion and cell elongation

By balancing nutritional components and increasing CO_2 fixation through prolonged stomata opening, the use of super absorbent polymers enhances cell membrane growth, leaf area index, leaf area duration, chlorophyll, and protein content, which contributes to the improvement in yield qualities of mustard [111, 112]. The increases in total photosynthetic pigments may be due to the role of eco-friendly hydrogel polymer in decreasing chlorophyll degradation or increasing chlorophyll biosynthesis due to supply of sufficient amount of water and nutrients to the plant in water deficit condition. Besides, carotenoid prevents the generation of singlet oxygen and protects plant from oxidative damage. By gently pumping water into the plant, hydrogel, according to Tongo et al. [110], improved photosynthesis. Additionally, the use of hydrogel polymers improved the water use effectiveness and CO_2 absorption rate of plants cultivated under drought stress [73].

Unfortunate, there is no researches deal with the effect of hydrogels on changes in different biochemical metabolites during plant life. Meanwhile, all the collected facts on beneficial effect of hydrogel on plant growth and productivity depends on their roles in increasing the capacity of water storage of soil [26, 113], reducing wasting water and nutrition materials of soil [24], reducing water evaporation from the surface of soil [26] and increasing the aeration of soil [25] caused the better growth of plants under water stress condition [27]. Results of this work indicated in Figure 6 and 7, showed the promoted effect of hydrogel on proline content, antioxidant system, and inhibiting effect on H_2O_2 and MDA in soybean plant irrigated every 7 days over those irrigated every 10 days. All of these facts concerned on hydrogel role in tolerating plant to water deficient and reflecting on enhancement antioxidant system of plant (non-enzymatic antioxidant and antioxidant enzymes) for detoxifying and neutralizing harmful ROS that produced under normal or stressed conditions .

Molecular markers

According to Mudibu et al. [114], ISSR is an effective marker type for locating polymorphisms in DNA sequences. Due to the use of lengthy primers, ISSR markers are very repeatable (16-25 mers). A high amount of genomic polymorphisms can be detected with ISSRs since they often contain a lot of polymorphic bands (up to 97) [115]. In genetics and plant breeding, DNA-based molecular markers have been incredibly useful [116]. Given the high annealing temperature of primers, high repetition, low cost, and available genomic data, ISSRs among DNA markers have an advantage [117]. However, Gaafar et al. [118] used five ISSR primers to evaluate the effect of different gamma ray doses on ISSR polymorphism of two Egyptian soybean varieties, and reported that there were variable amplified DNA bands detected by γ-radiation which might be produced by different types of DNA damages. Clara et al. [119] used different ISSR primers to determine the relationship and similarity among different cultivars of sunflower and reported that Primers HB-13 and HB-15 displayed the maximum polymorphism (100%). Moreover, Dawood et al. [120] used ISSR molecular markers to study the molecular changes chickpea as an effect of interaction between both proline and glycinebetaine with salinity, and they found that there were a variety of different types of polymorphic bands were detected as a response to salinity effects. Also, Liu et al. [121] decided that there were several Random Amplified Polymorphic (RAPD) markers linked with salt tolerance characteristics of different plant species. Moreover, Elsayed et al. [122] and El-Awadi et al. [123] studied the influence of salinity on growth and genetic range of broad bean and they obtained that all used primers provided polymorphic patterns among cultivars.

5. Conclusion

HPXa hydrogel were successfully prepared and characterized in terms of its physiochemical properties. HPXa showed greater swelling capacity. It could be decided that incorporating different levels of HPMC-Xanthan gum hydrogel as super absorbent polymers into the soil under irrigation every 7 days was more pronounced than those irrigated every 10 days. However, under two irrigation levels, HPMC-Xanthan gum hydrogel significantly increased most of soybean plant growth parameters, total photosynthetic pigment, proline, phenolic content, flavonoid, catalase, peroxidase, superoxide dismutase, glutathione reductase accompanied by significant decreases in MDA, and H_2O_2 relative to corresponding controls. It is worthy to mention that HPMC-Xanthan gum hydrogel have promising role in saving amount of water used in soybean agriculture via decreasing the number of irrigation along plant growth.

Future work

Developing better way to control the time of degradation of biodegradable polymers through blending with different molecular weight and different natural or synthetic polymers to get long acting biodegradable hydrogel.

6. Declaration: The authors declare the work is not published anywhere else.

7. Ethics approval and consent to participate

There is no need as clinical trials are not involved in study.

8. Consent for publication: The authors gave their consent for publication.

9. Availability of data and material: The data is available but not attached with manuscript.

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