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Environmental impacts of transgenic Bt rice and non-Bt rice cultivars in northern Iran

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ABSTRACT

In the future, rice production is likely to be impacted by climate change and associated risks including pest complexes and consumption of chemical inputs, thereby decreasing production at a regional scale and one of the major global concerns. In fact, among the biotic stresses, damage caused by insect pests, which can be categorized as either minor or major pests, can severely constrain the potential yield of rice. Hence, planting transgenic rice is considered a solution for reducing environmental and human health impacts. Therefore, in this research, life cycle assessment for environmental and human health impacts of transgenic and non-transgenic rice cultivars was considered. Hence, four transgenic lines (driven from back cross of Khazar cultivar with transgenic line of Tarom Molaii) along with conventional cultivars (non-transgenic parents) were cultivated under the standard of biosafety protocol in three isolated sites in north of Iran in 2016. In order to conducted life cycle assessment, first, the results of each site were analyzed separately, and since there were not differences among the impact categories and indices in different sites, the average for the results of the three sites are being presented. Results show that decreased insecticides application in transgenic cultivars lowers the need for labor, machineries, and fuel, thereby contributing to reduction in use of energy and greenhouse gases emission from construction, transportation and application of inputs during cultivation. Furthermore, most investigated impact categories were obtained high for Tarom Molaii and Khazar and less for three transgenic lines. In addition, the most important categories were non-renewable energy, global warming, aquatic eutrophication, aquatic acidification, terrestrial acid/nutri, land occupation, terrestrial ecotoxicity, ozone layer depletion, ionizing radiation, respiratory inorganics, respiratory organics, cumulative energy demand, ecological footprint, greenhouse gas protocol, water footprint, carcinogens and non-carcinogens. Therefore, according to the findings of this research, it was observed that the emission amount of environmental pollutants has a positive correlation with the consumption of inputs and field management practices.

1. Introduction

A major challenge of the 21st century is to achieve security in food supply under a changing climate and to roughly double food demand by 2050 compared to the present, the majority of which needs to be met by cereals, especially rice (Rotter et al., 2015). Agricultural parts especially rice production is considered environmental pollution in developing countries (Smith et al., 2007). Hence, it is of great necessity to evaluate the life-cycle of rice and its products to determine greenhouse gases emissions in order to reduce environmental pollutant (Habibi et al., 2019). Therefore, right decision-making process is one of the most important options for good management practice of paddy fields by considering conventional planting system and other opportunities (Nabavi-Pelesaraei et al., 2018).

Field-testing genetically modified crops provides scientists with an opportunity to collect information on environmental interactions and agronomic performance, which is critical to a full environmental safety assessment as required by regulatory authorities (Dastan et al., 2019). Therefore, in order to eliminate starvation and improve food security for the growing population in the world, it is necessary to increase food production, but, along with that, the adverse environmental impacts must be reduced. This requires the application of modern scientific findings and technologies. In so doing, cultivating transgenic plants is one of the options with several benefits (ISAAA, 2011). Since 1996, plants have been modified with short sequences of genes from *Bt.* (*Bacillus thuringiensis*) to express the crystal protein *Bt.* makes. In 2017, the area under the cultivation of GMO was reported to be 189.8 million hectares around the world (ISAAA, 2017).

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Rice is the staple food of Iranian people and more than half of the world's population and obviously affects feeding, income and job creation of people in the world, especially Iran (Pishgar-Komleh et al., 2011). Rice production has been facing serious challenges since 2000, making the international community concerned about the supply of food for people in the world. According to the investigations, the most important method to fight rice stem borer (*Chilo suppressalis* Walker) in Iran and also most regions in the world has been the chemical inputs (Ministry of jihad-e-agriculture of Iran, 2016). In this regard, every year, thousands of chemical pesticides resources are used in the paddy fields (almost half the pesticide used in paddy fields is against Lepidoptera) (FAO, 2011). Considering the role of rice stem borer (*Chilo suppressalis* Walker) in significant reduction of yield, the chemical control begins from the phase of nursery preparation, which causes severe environmental pollution and severe injury to humans' health (Alinia et al., 2000).

Environmental assessment is one of the accepted methods for achieving sustainable agricultural goals. Life cycle assessment (LCA) is an appropriate way to studying and investigating the environmental impact of producing a product in its whole life cycle in production systems (Iriarte et al., 2010). Various studies have been found in this regard. Habibi et al. (2019) by using LCA to assess 200 rice production fields (rice production systems including low-input, conventional and high-input in traditional and semi-mechanized planting methods in different paddy fields size levels) in Mazandaran and Guilan provinces, Iran reported that in both regions, all the impact categories and environmental pollutant were almost same and farmer's management practices are close to each other. Also, climate change (CC) in Amol and Rasht regions was 277.21 and 275.79 kg CO_2 eq, respectively. The most CC, global warming potential (GWP 100a) and cumulative energy demand (CED) in both regions were observed in high-input system for semi-mechanized method. Furthermore, the result for the impact categories of terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), agricultural land occupation (ALO), water depletion (WD), metal depletion (MD), and fossil depletion (FD) was similar to the CC, GWP, and CED where the highest amounts in both regions statistically went to high-input system, traditional planting method and small farms. Moreover, in both regions, high-input and conventional systems emitted higher heavy metals than low-input system. Furthermore, the most heavy metals emission in the air was achieved in small farm, and medium farm got the next rank. Using LCA, Mohammadi et al. (2015) assessed 82 rice paddy fields in northern Iran, and found that spring cultivation had a weaker environmental impact ("global warming, acidification, eutrophication, non-renewable energy demand and water depletion") than summer. The main cause for these results is lower consumption of inputs and higher paddy yield production in rice cultivation in springer compared to summer. He et al. (2018a) showed that organic rice production system had lower environmental impact compared to conventional system in sub-tropical China throughout the life cycle. They announced that chemical fertilizer and pesticide consumption were the main factors causing higher non-renewable energy depletion, GWP, soil toxicity, eutrophication potential, land occupation, acidification potential, water depletion, human toxicity potential, and aquatic toxicity potential in organic rice production system. Yodkhum et al. (2017) by using LCA methodology for organic rice production in Thailand reported that GHGs emission equals 0.58 kg CO_2 eq per kg of paddy which the main share was related to farm emission (83% of total GHGs emission), followed by land preparation (9%), harvesting (5%) and other stages (3%). Moreover, Nabavi-Pelesaraei et al. (2018) assessed 240 paddy fields in Guilan province, Iran. LCA finding demonstrated that rice production leads to 1166.09 kg CO_2 eq. emission per ton. They found that rice production is hotspot in terms of energy consumption, global warming, acidification, and eutrophication impact categories. Using LCA-ReCiPe method in Bangladesh, Jimmy et al. (2017) reported that the magnitude of impact per kg of paddy produced from the harvested field; a CO_2 eq emission of 3.15 kg as global warming potential, fossil depletion of 0.68 kg oil eq, a N eq emission of 0.0154 kg as marine eutrophication a P eq emission of 0.00122 kg as freshwater eutrophication, a 1,4-DCB-kg oil eq emission of 1.15 kg as human toxicity and use of 2.97 m^3 of water for irrigation purpose. They demonstrated that manufacture of fertilizer and pesticide also play a significant role in putting environmental load. Using LCA, Bacenetti et al. (2016) assessed 70 ha of organic rice production fields located in Lomellina of Italy, and found that CH_4 emissions from the flooded fields, compost production, nitrogen associated emissions and the mechanization of the paddy field practices were the main environmental hotspots for organic rice. Moreover, literature interview indicated that there are numerous studies about the environmental assessment for rice production in countries such as USA (Linquist et al., 2012), Japan (Koga and Tajima, 2011; Hokazono and Hayashi, 2012), China (Zhang et al., 2010), Italy (Zhang et al., 2010Blengini and Busto, 2009), and Taiwan (Yang et al., 2009). Similar studies were done based on LCA in order to make comparisons between the production systems of sugar beet (Tzilivakis et al., 2005), rice (Coltro et al., 2017; Fusi et al., 2014; Firouzi et al., 2018; Hokazono and Hayashi, 2012; Khoshnevisan et al., 2014; Nabavi-Pelesaraei et al., 2017; Nunes et al., 2016; Roy et al., 2005, 2009).

Several life cycle assessment have been found for GM crops. Bennett et al. (2006b), by using LCA to compare the environmental impact of production and feeding of GM and non-GM maize to broiler production in Argentina, revealed that there are both human health and environmental benefits of growing GM maize including lower impacts on global warming potential, human toxicity, ozone layer depletion and freshwater ecotoxicity. Similar studies were done based on LCA in order to comparison of conventional and GMO cultivars of sugar beet (Brentrup et al., 2001; Bennett et al., 2004; Bennett et al., 2006a). Moreover, there are several reports in the scientific literature showing that the use of GM crops can markedly reduce pesticide use and the environmental footprint of agriculture on the environment (Carpenter et al., 2002; Phipps and Park, 2002; Gianessi et al., 2003) Furthermore, Dale et al. (2002) demonstrated that GM crops are innately different from non-GM crops. The kinds of potential impacts of GM crops fall into classes familiar from the cultivation of non-GM crops (e.g., invasiveness, toxicity, or biodiversity). It is likely, however, that the novelty of some of the products of GM crop improvement will present new challenges and perhaps opportunities to manage particular crops in creative ways. Therefore, through LCA, very useful information can be obtained about the environmental impacts of different inputs on the production of transgenic and non-transgenic rice and their comparison with one another. To the best of our knowledge, LCA has not been applied so far to specifically assess the environmental impact of GM crops in Iran. Moreover, the LCA method for investigating transgenic rice has not yet been used in Iran and other countries to assess the environmental and human health impacts of producing transgenic plants, and this is the first study of this kind in Iran and some parts of the world. As well as, comparison of different methods of LCA has not yet been used by other researchers in the world. So, investigating transgenic and non-transgenic rice genotypes gives us valid data to assess and compare transgenic and no-transgenic rice genotypes in terms of safety and environmental risk. As a result, the purpose of this research is to investigate the environmental aspects of producing transgenic and non-transgenic rice genotypes by different life cycle assessment methods.

2. Materials and methods

2.1. Description of the site

Mazandaran and Guilan provinces are located in the northern part of the Alborz Mountains range and south of the Caspian Sea in northern Iran, west of the Mediterranean (Habibi et al., 2019). This survey was conducted in Amol region (in the western part of Mazandaran province), Sari region (in the central part of Mazandaran province) and

Rasht region (in the central part of Guilan province) in 2016. Rasht region is geographically situated at 37°, 30′ to 37°, 27′ N latitude and 49°, 27′ to 49°, 55′ E longitude. In addition, Amol region is geographically situated at 36°, 14′ to 36°, 29′ N latitude and 52°, 21′ to 52°, 38′ E longitude. Moreover, Sari region is geographically situated at 36°, 37′ to 36°, 42′ N latitude and 53°, 19′ to 53°, 01' E longitude. Experiments were done in isolated paddy fields, under the control of Rice Research Institute of Iran (RRII) in three sites in north of Iran in the provinces of Mazandaran and Guilan (Table 1) in 2016. The geographical coordinate and soil properties of three sites are shown in Table 1. The most important climatic parameters of three sites during the rice growth period are shown in Table 1.

In rice growing season (from April to September), its climate is temperate sub-humid. Harvest period of rice is usually during September, after which clover, canola or wheat is sown in a double cropping system. Some farmers after harvesting their rice product are transplanting rice or manage the residue for ratooning harvest. In rice fields of these regions, common used fertilizers included urea (46% N), triple super phosphate (48% P_2O_5), and sulfate potassium (48% K_2O), complete macro fertilizer (15% N, 8% P_2O_5 and 15% K_2O) and foliar application of complete macronutrient and micronutrients. Also, several pesticides such as insecticide, fungicide herbicide are used.

2.2. Description of experiment

This experiment included four transgenic rice lines (driven from the back cross of Khazar cultivars with the transgenic line of Tarom Molaii) containing the gene *cry*1Ab from the bacterium *Bt.* (*Bacillus thuringiensis*) with the transgenic line of Tarom Molaii (containing the gene for resistance against striped stem borer as the non-restorer parent) along with the non-transgenic cultivars. The diagram of breeding scheme of backcross lines is shown in Fig. 1. The profile of the genotypes is shown in Table 1. In addition, the pictures of field GM/non-GM rice along with the striped stem borer-induced DH and WH in the experiment are shown in Fig. 2.

Considering the climates of the regions, the seedlings were transplanted in 3–4 leaf stages. Considering the type of the cultivar, the transplanting operation was performed in the regions with similar situations. It was decided that the size of each plot should be $4 \times 7\,m^2$ and the planting density was 16 plants per square meter in a $25 \times 25\,cm^2$ arrangement. Nitrogen, phosphorous, and potassium fertilizers were applied according to the suggestions of the Iran Rice Research Institute of Iran (RRII) and after considering the result of soil analysis. All the phosphorus amount and one-third of the nitrogen fertilizer was used as basal in the paddy field preparation stage. Two-thirds of the nitrogen fertilizer was used as top-dressing in panicle initiation and the full-heading stages. Sixty percent of potassium fertilizer was used as basal, and the remaining amount was used as top-dressing in the tillering and the panicle-initiation stages (splitting to 20 percent at each stage).

The isolation conditions were met according to biosafety principles (standard of biosafety protocol) consisting of cultivation with distance from other paddy fields around, putting enough margin space around the field, harvesting the margin separately, and eliminating the plants remaining after the experiment. The experiment time and space distance were also considered based on biosafety principles. Crop protection practices, irrigation, weeding and fertilization, were done in the isolated experimental paddy field in each region. Stem borer eggs were collected from paddy fields, hatched in the laboratory, and cultured under the same environmental conditions, including a 16-h photoperiod (He et al., 2018b). Stem borer larva were maintained on germinated rice seeds (cv. Tarom Hashemi) in glass bottles at 28 ± 1 °C with 70–80% relative humidity, as described by He et al. (2017). The experimental plots were bordered by four rows of non-GM rice plants as protection. The experimental fields were not sprayed with chemical pesticides during the entire growth period to determine GM rice resistance to dead heart (DH) and white heads (WH) (Ling et al., 2016). Other crop management practices applied followed the standards of Iran's Biosafety Clearing-House protocol[1] and Standard Evaluation System (SES)[2] of the International Rice Research Institute (IRRI).

2.3. Life cycle assessment (LCA)

In this research, for life cycle assessment, first, the results of each region for rice genotypes were analyzed separately, and because there were no significant differences between the impact categories and the indices for different genotypes, the average of the three regions was considered and presented. LCA is carried out in four main phases: definition of goals and scope; analysis of inventory; impact assessment and interpretation.

2.3.1. Expression of the goals, scope and the functional unit

The goal of LCA in this research was to assess the environmental impact of producing transgenic and non-transgenic rice genotypes through LCA. The functional unit was based on producing one ton of paddy yield (at a water content of 12%). Considering that the paddy field had two outputs, a paddy yield and straw yield, the allocation of the environmental impacts (Rebitzer et al., 2004) was considered to be 90% paddy yield and 10% straw yield which are according to their economic value.

2.3.2. Life cycle inventory (LCI)

In this step, all resources and inputs used for transgenic and non-transgenic rice genotypes and also all amounts of the pollutants emitted to environment due to the use of different types of inputs in each site were determined and listed separately. In the listing step, these items were considered: (i) infrastructures, comprising construction, maintenance and depreciation of machinery and buildings (shelters for machinery); (ii) all agricultural operations including bed preparation cultivation, fertilization, protection, irrigation, harvest, transportation supply and consumption of fuel for the operations; and (iii) producing the fertilizers and pesticides and transferring them. The result of the step of listing is a list of inputs to the field, outputs, and field emissions.

2.3.3. Life cycle impact assessment (LCIA)

In this step, considering the importance of environmental issues in the production of transgenic rice genotypes, all important and effective indices were estimated. Evaluating the LCA results based on different methods and impact categories, provide and help the readers to have more comprehensive results and view about our results. After all the investigations on different models, the life cycle assessment and the comparison of the results were done based on Impact 2002 + model, and the total indices of pollutants emissions were calculated. In order to have an accurate and complete life cycle assessment of the environmental impacts in the next step with other models (ReCiPe 2016, Impact, 2002+, Ecopoints 97, Cumulative Energy Demand, Ecological footprint, Ecosystem Damage Potential, Greenhouse Gas Protocol, Water footprint, EPS, 2000, LCI result and TRACI); all indices of environmental pollutants emissions were calculated and the LCA was completely done; thereafter, the results obtained from each model were compared and assessed for eight rice genotypes.

In the impact assessment step, first, the impacts each of the substances emitted into the environment has is determined, the amount of which was determined in the previous step, and in what impact step or categories should it go to. Then, this emission converts into an equivalent unit for the impact category. For instance, the emitted gases

[1] http://ir.biosafetyclearinghouse.net/database/record.shtml?documentid=108923.

[2] http://www.knowledgebank.irri.org/images/docs/rice-standard-evaluation-system.pdf.

Table 1
Description of geographical coordinate, soil properties (0–30 cm) and climate parameters of three rice production sites. As well as description of name, origin and other characteristics of rice genotypes.

Description	Sari (Mazandaran province)		Amol (Mazandaran province)		Rasht (Guilan province)		
Geographical coordinate	36°39′22.52″N 53°9′42.55″E		36°23′59.24″N 52°31′37.55″E		37°13′28.78″N 49°38′57.85″E		
Soil properties	Sari (Mazandaran province)		Amol (Mazandaran province)		Rasht (Guilan province)		
Soil texture	Clay loam		Silt clay		Clay		
EC (dSm^{-1})	0.92		0.96		0.98		
pH	7.25		7.58		6.60		
Organic matter (%)	2.46		2.11		2.31		
Phosphorus ($mg\ kg^{-1}$)	12.50		8.90		19.60		
Potassium ($mg\ kg^{-1}$)	185		208		205		
Climate parameters	Sari (Mazandaran province)		Amol (Mazandaran province)		Rasht (Guilan province)		
	Experiment period	Mean 15 years	Experiment period	Mean 15 years	Experiment period	Mean 15 years	
Minimum temperature (°C)	18.4	18.3	18.9	18.5	13.8	17.6	
Maximum temperature (°C)	28.4	25.2	27.7	26.9	33.6	26.5	
Mean temperature (°C)	23.4	22.8	23.3	32.2	22.3	22.1	
Evaporation (mm)	143.7	147.6	109.5	120.8	99.6	121.4	
Rain (mm)	52.5	89.0	50.8	93.4	77.7	60.7	
Mean humidity (%)	74.7	73.5	75.8	77.5	78.7	78.0	
Mean sunshine hours	221.1	208.8	187.6	182.7	186.2	213.9	
Solar radiation ($MJ\ m^{-2}\ d^{-1}$)	19.3	19.5	17.8	17.9	17.7	18.1	
Genotype	Back cross (♀ × ♂)	Description	Plant height	Yield condition	Maturity	Type	Origin
Nemat	Improved	Non-transgenic	Semi-dwarf	High yield	Late maturing	Improved	Iran
Khazar	Improved	Non-transgenic	Semi-dwarf	Medium yield	Medium maturing	Improved	Iran
Tarom-Hashemi	Local	Non-transgenic	Tall	Low yield	Early maturing	Local	Iran
Tarom-Molaii	Local	Transgenic	Tall	Low yield	Early maturing	Local	Iran
KHT_2	Khazar/Bt. Tarom- Molaii	Transgenic	Semi-dwarf	Medium yield	Medium maturing	Improved	Iran
KHT_3	Khazar/Bt. Tarom- Molaii	Transgenic	Semi-dwarf	Medium yield	Medium maturing	Improved	Iran
KHT_4	Khazar/Bt. Tarom- Molaii	Transgenic	Semi-dwarf	Medium yield	Medium maturing	Improved	Iran

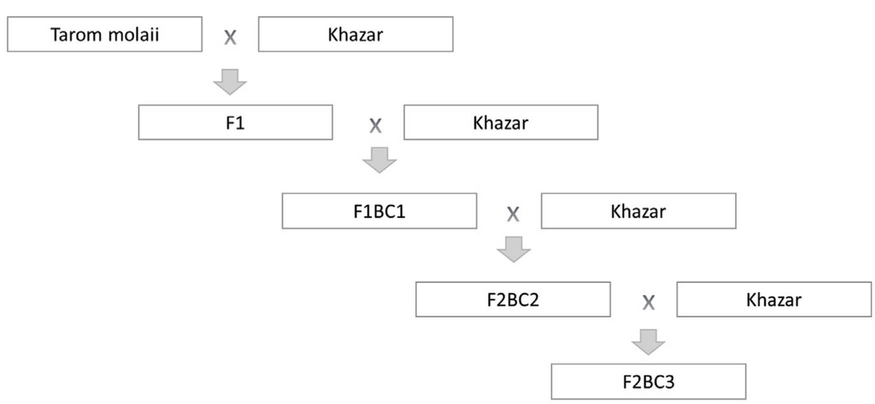

Fig. 1. The diagram of breeding scheme of backcross lines.

of CO_2, N_2O and CH_4 have impacts on global warming, but their potentials for making climate changes are different, so that a kilogram of N_2O and CH_4 has the greenhouse effect of 310 and 25 kg of CO_2, respectively (Brentrup et al., 2004b). In order to evaluate the environmental impacts and to accurately interpret the inputs and outputs, the steps of characterization in the LCA was taken.

Characterization step is a necessary step in LCA. According to ISO, impact categories are defined depending on the study type. In this research, by using different models of LCA in the software SimaPro8.2.3, numerous impact categories were estimated and compared for producing one-ton paddy yield of transgenic and non-transgenic rice genotypes with the related impact. The most important impact categories in this research were non-renewable energy, global warming, aquatic eutrophication, aquatic acidification, terrestrial acid/nutria, land occupation, terrestrial ecotoxicity, aquatic ecotoxicity, ozone layer depletion, ionizing radiation, respiratory inorganics, respiratory organics, cumulative energy demand, ecological footprint, greenhouse gas protocol, water footprint, carcinogens and non-carcinogens (Brentrup et al., 2004b). In the last step of a LCA, the results obtained for comparing the environmental impacts of producing transgenic and non-transgenic rice genotypes in the three sites were analyzed according to the standard systems.

2.3.4. Damage assessment

Since there are many indices for the impact category, it is hard to interpret the research results. In order to simplify the results', interpretation, a process of grouping has been used in some of the methods used in LCA, such as Impact 2002+, ESP 2000, eco-indicator, etc. In these methods, the impact categories were defined close to one of the groups of the final point (human health, ecosystem quality, climate

Fig. 2. Dead heart of striped stem borer insect (*Chilo suppressalis* Walker) (A), white heads of striped stem borer insect (*Chilo suppressalis* Walker) (B), start of tillering stage (C), middle of tillering stage (D), initial heading stage (E), full heading stage (F), 50% of flowering stage (G) and physiological maturity stage (H) in the experimental paddy fields.

change, resources and biodiversity) for reaching the optimal environmental relationship. The impact categories belonging to the final categories all share similar units, so they can be added easily and the final number shows the measure of the group of the final impact.

2.3.5. Interpretation

One of the aims of LCA is to provide comprehensive information for decision makers. To achieve this goal, LCA results of a study must be interpreted. In this step, the LCA results of different scenarios (production of GM and non-GM rice cultivars) were evaluated and compared.

2.3.6. Eco-index

In the final step, some kind of environmental index named eco–index was calculated, which is the final criterion of LCA (Brentrup et al., 2004b):

$$EcoX = \sum Ni \times W_i$$

where EcoX is the environmental Eco-index per functional unit; Ni is the normalized measure for every impact categories; Wi is the weight for every amount of Ni. The larger the environmental index indicates, the further is potential damage to the environment.

3. Results

3.1. ReCiPe method

Climate change impact category was estimated based on the mass emission of N_2O, CH_4 and CO_2. These emissions are transferred into kg CO_2 eq. the average CC was 640.32 kg CO_2 eq (Table 2). The finding revealed that the highest CC was estimated Khazar (785.87 kg CO_2 eq) and Tarom Molaii was ranked next. The GM lines derived from Khazar (KHT_2, KHT_3 and KHT_4) showed lowest CC (553.35, 554.81 and 553.62 kg CO_2 eq). The result for the impact category of ozone depletion was similar to those for CC where the highest amounts went to Khazar cultivar and the lowest amount was observed for KHT_2, KHT_3 and KHT_4 (Table 2).

The impact category of terrestrial acidification (TA) of GM lines derived from Khazar (KHT_2, KHT_3 and KHT_4) was lower than non-GM cultivars (Nemat, Khazar, and Tarom Hashemi) and GM Tarom Molaii. Moreover, TA of Khazar (4.83 kg SO_2 eq) and Tarom Molaii (4.64 kg SO_2 eq) cultivar was higher than other cultivars (Table 2). As shown in Table 2, the average freshwater eutrophication (FE) and marine eutrophication (ME) in GM and non-GM cultivars were 0.018924 kg P eq and 0.024977 kg N eq. The impact category of FE and ME in Khazar cultivar was significantly higher than other cultivars. Both impact categories for GM lines derived from Khazar were lower than other GM and non-GM cultivars (Table 2). The average amount of human toxicity, terrestrial ecotoxicity, freshwater ecotoxicity and marine ecotoxicity were 272.96, 0.108325, 12.78 and 12.08 kg 1,4-DB-eq, respectively (Table 2). The highest amounts were assigned to Tarom Molaii and Khazar. The GM cultivars of Khazar (KHT_2, KHT_3 and KHT_4) showed less human toxicity, terrestrial ecotoxicity, freshwater ecotoxicity and marine ecotoxicity (Table 2). The result for the impact category of photochemical oxidant formation was similar to that for other impact categories, where the highest amount was observed in Khazar and Tarom Molaii. Nemat (non-GM cultivar) ranked third. Lowest photochemical oxidant formation was observed in GM cultivars derived from Khazar (Table 2). In addition, the average particular matter formation and ionizing radiation were 1.93 kg PM10 eq and 76.26 kBq U235 eq, respectively. The highest amounts were assigned to Khazar and Tarom Molaii got ranks next. The GM cultivars of Khazar (KHT_2, KHT_3 and KHT_4) showed less amounts (Table 2).

As shown in Table 2, the average agricultural land occupation (ALO) and urban land occupation (OLO) were 73.95 and 24.71 m^2a, respectively. This result showed that ALO and OLO in KHT_2, KHT_3 and KHT_4 are less and almost the same. The most amount of ALO and OLO belongs to Khazar and Tarom Molaii ranked next, and Nemat got ranked third. Natural land transformation (NLT) amount in KHT_2, KHT_3 and KHT_4 was same and lower than other cultivars. NLT of Khazar and Tarom Molaii was higher than others (Table 2). Regarding the net impact category, the average amount of water depletion (WD) was 60.49 m^3. The maximum WD was achieved for Khazar and Tarom Molaii got ranked next. Furthermore, WD of GM lines derived from Khazar was less than other cultivars (Table 2). According to the results of Table 2, the average metal depletion (MD) equals 86.38 kg Fe eq, moreover fossil depletion (FD) equals 225.19 kg oil eq. In terms of MD and FD impact categories, KHT_2, KHT_3 and KHT_4 showed the lowest amount and Nemat cultivar got the next rank. MD and FD for Khazar cultivar were slightly higher than Tarom Molaii, but these impact categories were highest in these cultivars (Table 2).

3.2. Impact 2002 + model

The measures for environmental assessment indices for Impact 2002 + model are given in Table 3. Among different cultivars, the highest production of carcinogens and non-carcinogens per a ton of paddy yield were observed in the cultivars Khazar and Tarom Molaii, respectively. The cultivars Tarom Hashemi and Nemat earned third and fourth ranks. All the Khazar transgenic lines with almost equal amounts

Table 2
Characterization impact indices in ReCiPe method for transgenic and non-transgenic rice genotypes.

Impact category	Unit	Nemat	Khazar	Hashemi	Molaii	KHT_2	KHT_3	KHT_4	Mean	SE	CV (%)
Climate change	kg CO_2 eq	623.14	785.87	639.54	771.88	553.35	554.81	553.62	640.32	38.17	15.77
Ozone depletion	kg CFC-11 eq	0.000148	0.000187	0.000146	0.000109	7.83E-05	7.85E-05	7.83E-05	0.000118	1.63E-05	36.59
Terrestrial acidification	kg SO_2 eq	3.88	4.83	3.93	4.64	3.31	3.32	3.31	3.89	0.240535	16.36
Freshwater eutrophication	kg P eq	0.274275	0.35769	0.300508	0.355692	0.245816	0.246468	0.24594	0.289484	0.018924	17.30
Marine eutrophication	kg N eq	0.276462	0.36778	0.339933	0.381952	0.234242	0.234863	0.234359	0.295656	0.024977	22.35
Human toxicity	kg 1,4-DB eq	265.54	338.20	279.27	330.03	232.32	232.94	232.44	272.96	17.25	16.72
Photochemical oxidant formation	kg NMVOC	2.21	2.85	2.34	2.81	1.99	1.99	1.99	2.31	0.142146	16.25
Particulate matter formation	kg PM10 eq	1.86	2.36	1.93	2.34	1.66	1.67	1.66	1.93	0.11617	15.95
Terrestrial ecotoxicity	kg 1,4-DB eq	0.12	0.15	0.118491	0.121679	0.085101	0.085327	0.085144	0.108325	0.008907	21.76
Freshwater ecotoxicity	kg 1,4-DB eq	12.10	15.68	12.95	15.64	11.03	11.06	11.04	12.78	0.788165	16.31
Marine ecotoxicity	kg 1,4-DB eq	11.50	14.80	12.20	14.74	10.43	10.46	10.44	12.08	0.738426	16.17
Ionising radiation	kBq U235 eq	74.56	94.32	76.70	91.47	65.51	65.68	65.54	76.26	4.64	16.09
Agricultural land occupation	m^2a	66.01	88.22	78.68	96.21	62.78	62.95	62.81	73.95	5.24	18.75
Urban land occupation	m^2a	21.97	29.13	25.74	32.22	21.34	21.40	21.35	24.74	1.68	17.93
Natural land transformation	m^2	0.15	0.176621	0.140748	0.172037	0.126197	0.126532	0.126261	0.145075	0.008164	14.89
Water depletion	m^3	944.86	1255.08	1020.28	1289.23	929.62	932.09	930.09	1043.04	60.49	15.34
Metal depletion	kg Fe eq	81.60	105.95	87.58	105.58	74.57	74.77	74.61	86.38	5.32	16.29
Fossil depletion	kg oil eq	221.92	276.80	223.26	269.30	194.81	195.33	194.91	225.19	13.23	15.55

Table 3
Characterization impact indices in Impact (2002) + method for transgenic and non-transgenic rice genotypes.

Impact category	Unit	Nemat	Khazar	Tarom Hashemi	Tarom Molaii	KHT_2	KHT_3	KHT_4	Mean	SE	CV (%)
Carcinogens	kg C_2H_3Cl eq	17.78	23.12	18.85	22.88	16.37	16.42	16.38	18.83	1.13	15.90
Non-carcinogens	kg C_2H_3Cl eq	15.95	20.78	17.17	21.08	14.91	14.95	14.92	17.11	1.03	15.98
Respiratory organics	kg C_2H_4 eq	0.3183	0.4090	0.3322	0.3991	0.2868	0.2876	0.2870	0.3314	0.02	15.90
Respiratory inorganics	kg PM2.5 eq	1.09	1.40	1.14	1.38	0.9884	0.9910	0.9889	1.14	0.07	15.85
Ionizing radiation	Bq C-14 eq	7598	9617	7824	9332	6680	6697	6683	7776	473	16.10
Ozone layer depletion	g CFC-11 eq	0.1452	0.1836	0.1433	0.1080	0.0773	0.0775	0.0774	0.1160	0.02	36.39
Aquatic ecotoxicity	kg TEG water	51985	66783	56854	67094	45681	45802	45704	54272	3625	17.67
Terrestrial ecotoxicity	kg TEG soil	24163	31322	25632	31757	22703	22763	22714	25865	1520	15.54
Terrestrial acid/nutri	kg SO_2 eq	12.45	15.54	12.66	15.17	10.85	10.87	10.85	12.63	0.76	15.97
Land occupation	m^2org.arable	37.12	50.77	49.22	61.57	37.10	37.20	37.12	44.30	3.68	21.99
Aquatic acidification	kg SO_2 eq	4.04	5.05	4.12	4.86	3.47	3.47	3.47	4.07	0.25	16.39
Aquatic eutrophication	kg PO_4 P-lim	0.2944	0.3841	0.3415	0.3946	0.2549	0.2556	0.2551	0.3115	0.02	19.83
Global warming	kg CO_2 eq	591.36	743.64	601.89	727.06	524.27	525.66	524.53	605.49	35.72	15.61
Non-renewable energy	MJ primary	10913	13666	11038	13280	9591	9616	9595	11100	657	15.66
Mineral extraction	MJ surplus	69.90	90.14	74.98	91.28	64.13	64.30	64.16	74.13	4.54	16.20

in carcinogens and non-carcinogens stood in next ranks. The measures for respiratory inorganic's and respiratory organics' impact categories in transgenic and non-transgenic cultivars were not significant. Tarom Molaii cultivar had more respiratory organics and respiratory inorganics than Tarom Hashemi because paddy yield of Tarom Molaii was less than that of Tarom Hashemi (Table 3).

The highest amount of ionizing radiation belonged to Khazar and Tarom Molaii cultivars. Tarom Hashemi and Nemat cultivars with producing 7824 and 7598 Bq C-14 eq of ionizing radiation stepped third and fourth ranks. Ionizing radiation emission in three Khazar transgenic lines was less than non-transgenic cultivars that all transgenic lines got same rank. According to Table 3, regarding ionizing radiation Khazar ranked first and Tarom Molaii, Tarom Hashemi and Nemat stood next, respectively. The transgenic lines of KHT_2, KHT_3 and KHT_4 had the least ionizing radiation. Considering the impact category of ozone layer depletion, Khazar cultivar had the highest amount and Nemat and Tarom Hashemi cultivars stood second rank. Tarom Molaii cultivar with 0.1080 g CFC-11 eq stood next rank. The three Khazar transgenic lines had the least ozone layer depletion, and all were at the same level (Table 3). According to the findings of Table 3, Khazar and Tarom Molaii cultivars have the highest scores in aquatic eco-toxicity and terrestrial eco-toxicity impact categories. Non-transgenic Nemat and Tarom Hashemi cultivars got the next rank in terms of aquatic aquatic eco-toxicity and terrestrial eco-toxicity. Considering the terrestrial acid/nutri, transgenic lines showed lower levels. Tarom Molaii and Khazar cultivars showed more terrestrial acid/nutria than other cultivars (Table 3).

In terms of land occupation impact category, Tarom Molaii cultivar got the first place and Khazar and Tarom Hashemi cultivars got the next places. The transgenic lines of Khazar showed 26.7% less land occupation than their non-transgenic parents. The highest aquatic acidification belonged to Khazar and Tarom Molaii cultivars, and Tarom Hashemi and Nemat cultivars stood third and fourth ranks. The lowest aquatic acidification belonged to the transgenic lines derived from Khazar, compared to other cultivars (Table 3). Also, the impact category of aquatic eutrophication in transgenic lines was lower than non-transgenic ones. The highest aquatic eutrophication belonged to Khazar and Tarom Molaii cultivars (Table 3). The share of NH_3 in acidification potential was far more than that of NO_2 and SO_2. The NH_3 emission resource is urea fertilizer. Ammonia sublimation has an important impact on the formation of the environmental impacts of eutrophication and acidification. The release of NH_3 in sublimation from urea is a physical and chemical process, and it is more sensitive than N_2O to the management of fertilizer consumption. The emission of NH_3 from urea fertilizer is more than other fertilizers, and, unfortunately, urea fertilizer is excessively used in Iran, and there is little use of other sources of fertilizers.

The highest global warming was obtained for Khazar and Tarom Molaii cultivars, respectively. The lowest global warming was observed for three Khazar transgenic lines. The transgenic lines from Khazar showed 29.5% less global warming than their non-transgenic parent. The results for the impact category of non-renewable energy were similar to the ones for global warming, where the highest amounts went to Khazar and Tarom Molaii cultivars. The transgenic lines of Khazar showed 29% less mineral extraction than their non-transgenic parent (Table 3). The reason for higher energy consumption and global warming in non-GM cultivars can be attribute to their dependence on inputs and higher energy consumption for production, and these inputs are used without any regard to environmental issues. The results for comparing the input energies and global warming potential caused by it demonstrated that there was a direct correlation between input energies and global warming potential caused by it.

3.3. Ecopoints 97 model

The findings of Table 4 were output of Ecopoints 97 method with impact categories of heavy metals' emission and other environmental pollutants in air, water and soil, which showed that the heavy metals emitted in air (Pb, Cd, Zn and Hg) were less in transgenic lines than in non-transgenic cultivars (Table 4). Also, the heavy metals emitted in water (Cr, Zn, Cu, Cd, Hg, Pb and Ni) were less in transgenic cultivars than non-transgenic lines. Moreover, pollutants emitted from soil (nitrate, metals and pesticide) in transgenic lines derived from Khazar were less than their non-transgenic parent. The release of nitrate in non-transgenic Khazar cultivar was 35.3% higher than transgenic cultivar derived from Khazar. Furthermore, nitrate emission in Nemat cultivar was 3.11–7.33%. Nitrate emitted for Tarom Molaii and Tarom Hashemi cultivars was 875.47 and 694.04 g, respectively. The highest pesticide emission belonged to Tarom Molaii cultivar, and Tarom Hashemi and Khazar cultivars stood second and third ranks (Table 4). According to the findings of Table 4, the least NH_3 emission was observed for KHT_2, KHT_3 and KHT_4. The most NH_3 emission was observed for Khazar cultivar (Table 4). With a group comparison between transgenic and non-transgenic cultivars, it was observed that transgenic cultivars have less emission of heavy metals into water, air and soil, and the reason was less inputs. In fact, the amount of heavy metals into water and soil was calculated based on the annual estimation of these elements' deposit and also their entrance into soil through fertilizers, pesticides, seeds and deposit and their separation from soil by product harvest, leaching and soil erosion.

3.4. ESP 2000 model

It is observed in Fig. 3a that based on impact category of crop

Table 4
Characterization impact indices in Ecopoints 97 method for transgenic and non-transgenic rice genotypes.

Impact category	Unit	Nemat	Khazar	Tarom Hashemi	Tarom Molaii	KHT_2	KHT_3	KHT_4	Mean	SE	CV (%)
NH_3	g	163.85	183.08	142.63	175.62	131.92	132.27	131.98	151.62	8.37	14.61
Pb (air)	g	0.7613	0.9570	0.7882	0.9567	0.6782	0.6800	0.6786	0.7857	0.0471	15.88
Cd (air)	g	0.1038	0.1204	0.0942	0.1150	0.0860	0.0863	0.0861	0.0988	0.0055	14.64
Zn (air)	g	1.28	1.65	1.40	1.70	1.17	1.17	1.17	1.36	0.0867	16.84
Hg (air)	g	0.0268	0.0350	0.0286	0.0335	0.02375	0.0238	0.0237	0.0279	0.0018	17.02
P	g	33.91	45.33	42.17	45.22	27.08	27.15	27.09	35.42	3.28	24.43
N	g	63.58	80.11	61.30	37.10	26.92	26.99	26.93	46.13	8.27	47.45
Cr (water)	g	4.64	6.07	5.07	6.23	4.35	4.37	4.36	5.01	0.3094	16.33
Zn (water)	g	52.10	66.70	56.03	66.66	46.11	46.24	46.14	54.28	3.50	17.03
Cu (water)	g	60.22	78.18	64.52	78.92	55.82	55.97	55.85	64.21	3.89	16.03
Cd (water)	g	0.8025	1.02	0.8777	1.06	0.7179	0.7198	0.7183	0.8452	0.0551	17.25
Hg (water)	g	0.1156	0.1525	0.1385	0.1713	0.1101	0.1104	0.1101	0.1298	0.0093	19.05
Pb (water)	g	1.67	2.14	1.85	2.23	1.51	1.51	1.51	1.77	0.1162	17.33
Ni (water)	g	25.25	32.84	27.14	32.81	23.15	23.22	23.17	26.80	1.65	16.30
Nitrate (soil)	g	429.19	619.05	694.04	875.47	457.25	458.46	457.48	570.13	63.59	29.41
Metals (soil)	g Cd eq	0.0178	0.0239	0.0209	0.0262	0.0175	0.0175	0.0176	0.0202	0.0014	17.77
Pesticide (soil)	g act.subst.	0.8438	1.19	1.27	1.59	0.8704	0.8728	0.8709	1.07	0.1085	26.76
Waste	g	121450	159957	146377	181626	115847	116155	115905	136760	9970	19.29
Waste (soil)	g	11.89	15.40	12.57	14.84	10.59	10.61	10.59	12.36	0.7713	16.52

growth capacity, Khazar and Tarom Molaii cultivars stood first and second ranks. This index for Tarom Hashemi cultivar was 1.68 kg. Based on impact category of soil acidification, Khazar and Tarom Molaii cultivars had the highest amount. The least soil acidification related to transgenic lines derived from Khazar. Based on impact category of depletion of reserves and species extinction, Khazar and Tarom Molaii cultivars got the highest amount. Nemat and Tarom Hashemi cultivars stood next ranks. Furthermore, in other impact categories (life expectancy, severe morality, morbidity, severe nuisance and nuisance) Khazar and Tarom Molaii cultivars stood higher than others, and three transgenic lines had the least amounts (Fig. 3a).

3.5. LCI result model

Different impact categories are assessed in this method (Fig. 3b). Tarom Molaii cultivar had the highest emission of carbon monoxide, carbon, biogenic, fixed and dinitrogen monoxide, respectively. Tarom Hashemi cultivar stood second rank for these three impact categories. Khazar and Nemat cultivars stood next ranks with almost equal amounts. Although all transgenic and non-transgenic cultivars had no significant difference in terms of these pollutant emissions, Tarom Molaii shows much fewer these pollutant emissions. For methane emission, Tarom Molaii and Tarom Hashemi cultivars were first and second ranks. Methane emission in Khazar and its transgenic lines were

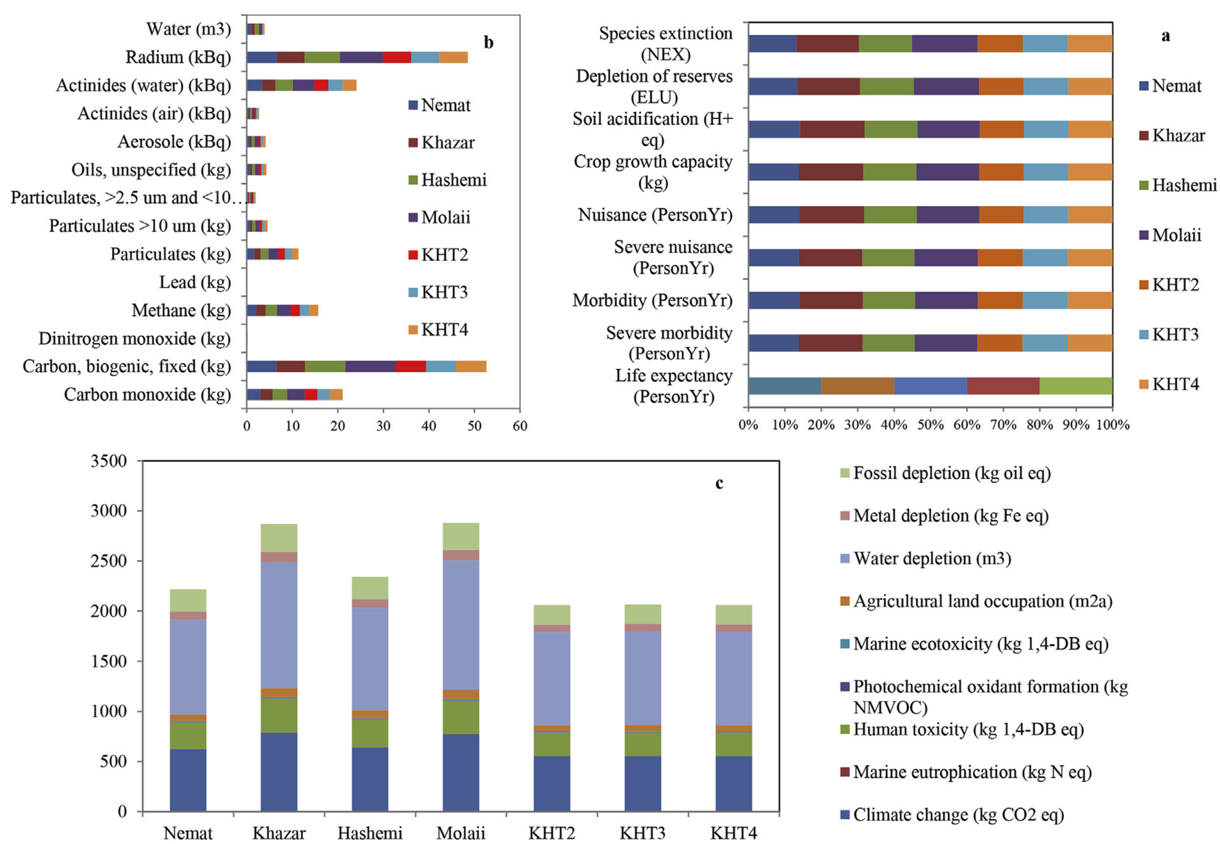

Fig. 3. Assessment of transgenic and non-transgenic rice genotypes by EPS 2000 method (A), LCI result method (B), and TRACI method (C).

equal. Lead emission in Tarom Molaii and Tarom Hashemi cultivars was higher than other cultivars. Lead emission in Khazar cultivar and its transgenic lines were obtained less than other cultivars (Fig. 3ab). The emission of other resources observable in Fig. 3b was higher in Tarom Molaii than other cultivars. Moreover, emission of other resources among all cultivars was not significant.

3.6. TRACI model

In TRACI model, three impact categories of smog, respiratory effects and fossil fuel depletion were assessed. The highest emission of smog belonged to Tarom Molaii cultivar, and Tarom Hashemi and Nemat cultivars came second and third ranks. Khazar cultivar and its three transgenic lines had equal smog emission. The least smog emission belonged to the three transgenic lines derived from Khazar. In terms of respiratory effects, Tarom Molaii cultivar stood first rank, and Tarom Hashemi and Nemat cultivars became second and third ranks. Khazar cultivar and its transgenic lines had the least respiratory effects, but difference between them was not significant. Fossil fuel depletion was statistically equal in Khazar cultivar and its three transgenic lines. In Tarom Molaii and Tarom Hashemi cultivars, fossil fuel depletion was 1268.77 and 1052.85 MJ surplus, respectively (Fig. 3c).

3.7. Cumulative energy demand model

According to the findings of Table 5, for the emission of non-renewable, fossil, Tarom Molaii cultivar stood first rank. Tarom Hashemi and Nemat cultivars took the next ranks with 9960 and 9896 MJ, respectively. The least non-renewable, fossil belonged to three transgenic lines of Khazar. From renewable water perspective, Tarom Molaii cultivars (400 MJ) got first rank, and Tarom Hashemi cultivars stood in second rank (Table 5). With group comparison between cultivars, regarding all the indices presented in Table 5, it was found out that Khazar is almost the same as transgenic lines. Results in Table 5 revealed that in terms of Energy Demand, Ecological footprint, Greenhouse Gas Protocol and Water footprint methods Tarom Molaii stood in first rank. Tarom Hashemi and Nemat cultivars stood second and third rank. Non-renewable energies are ecologically very important, as the source of non-renewable energies is mainly fossil fuels and relying on this resource in the future brings many risks.

3.8. Ecological footprint model

In this method, the three impact categories of carbon dioxide, nuclear and land occupation were assessed based on m^2a. For these three impact categories, Tarom Molaii cultivar had the highest amount, and Tarom Hashemi cultivar stood second rank. Khazar and Nemat cultivars took the third and fourth ranks with no significant difference compared with the three Khazar transgenic lines (Table 5).

3.9. Ecosystem Damage Potential model

The two impact categories of linear, land occupation and linear, land transformation were assessed in this method. Results showed that regarding both indices, there was not a significant difference between the three Khazar transgenic lines and Khazar and Nemat non-transgenic cultivars. But these two impact categories were higher in Tarom Molaii and Tarom Hashemi cultivars than other cultivars (Table 5).

3.10. Greenhouse gas protocol model

According to the findings of Table 5, the emission of fossil CO_2 eq was equal in Khazar cultivar and its transgenic lines. The emission of fossil CO_2 eq in Tarom Molaii and Tarom Hashemi cultivars was 754 and 625 kg CO_2 eq. In terms of emission of biogenic CO_2 eq and CO_2 eq from land transformation, Tarom Molaii and Tarom Hashemi cultivars stood on top. There was no difference between Khazar cultivar and its transgenic lines in terms of emission of biogenic CO_2 eq and CO_2 eq from land transformation. Tarom Molaii and Tarom Hashemi cultivars got higher ranks than other cultivars. In terms of biogenic CO_2 eq and CO_2 eq from land transformation there was not significant between Khazar cultivar and its transgenic lines. For CO_2 uptake, Tarom Molaii cultivar stood on top and Tarom Hashemi cultivar got the second rank. Regarding CO_2 uptake, no significant difference was observed among Khazar, Nemat and three transgenic lines derived from Khazar (Table 5).

3.11. Water footprint model

In this method, three impact categories of human health, ecosystem quality, and resources were assessed. In terms of human health based on DALY, no difference was observed among the cultivars. Regarding ecosystem quality, Tarom Molaii had the least amount, and Tarom

Table 5
Characterization impact indices in Cumulative Energy Demand, Ecological footprint, Greenhouse Gas Protocol and Water footprint methods for transgenic and non-transgenic rice genotypes.

Cumulative Energy Demand	Unit	Nemat	Khazar	Tarom Hashemi	Tarom Molaii	KHT_2	KHT_3	KHT_4	Mean	SE	CV (%)
Non-renewable, fossil	MJ	9896	8667	9960	12009	8686	8709	8691	9117	470.51	13.08
Renewable, water	MJ	310.08	284	332	400	284	285	285	311	16.31	13.85
Ecological footprint											
Carbon dioxide	m^2a	1496	1317	1515	1832	1325	1329	1326	1449	71.64	13.08
Land occupation	m^2a	122.79	113.43	137.34	168.42	115.61	115.91	115.66	127.02	7.57	15.76
Ecosystem Damage Potential											
linear, land occupation	points	46.89	43.26	52.20	63.78	43.91	44.03	43.93	48.29	2.84	15.56
linear, land transformation	points	2.61	2.44	3.37	4.22	2.55	2.55	2.55	2.90	0.2501	22.83
Greenhouse Gas Protocol											
Fossil CO_2 eq	kg CO_2 eq	614	542	625	754	544	545	544	582	35.63	16.19
Biogenic CO_2 eq	kg CO_2 eq	27.12	25.73	34.48	41.76	25.70	25.77	25.71	29.47	2.38	21.36
CO_2 eq from land transformation	kg CO_2 eq	1.03	0.95	1.17	1.42	0.96	0.96	0.96	1.06	0.0663	16.48
CO_2 uptake	kg CO_2 eq	42.73	40.51	53.50	65.54	41.09	41.20	41.11	46.53	3.61	20.54
Water footprint											
Human Health	DALY	0.0007	0.0007	0.0008	0.0010	0.0007	0.0007	0.0007	0.0006	0.000008	37.55
Ecosystem Quality	species*year	8.84	8.25	9.55	1.21	8.70	8.73	8.71	7.71	1.09	37.51
Resources	$ surplus	177.84	166.01	192.35	243.06	175.17	175.64	175.26	186.48	9.88	14.02

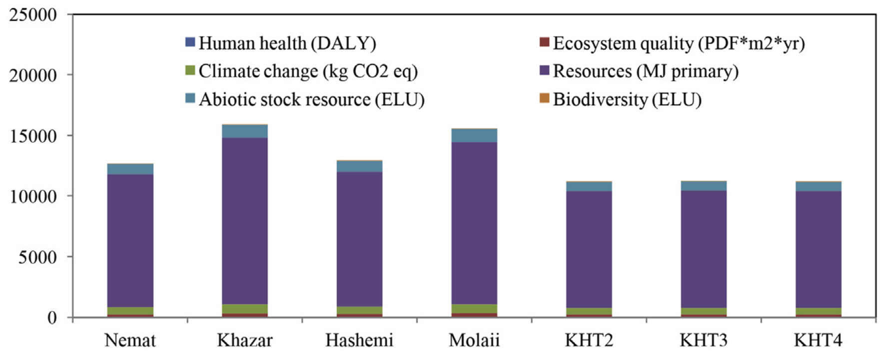

Fig. 4. Damage assessment of transgenic and non-transgenic rice genotypes by Impact (2002) + method.

Hashemi had the most, while the transgenic lines of KHT_2, KHT_3 and KHT_4 stood higher than non-transgenic Khazar. Regarding the index of resources, Tarom Molaii cultivar stood higher than other cultivars with a significant difference. Tarom Hashemi cultivar stood second rank. The three Khazar transgenic lines and Khazar cultivars did not have a significant difference regarding resources (Table 5).

3.12. Damage assessment

According to the findings of Fig. 4, it is observed that regarding human health, Tarom Molaii and Khazar cultivars had the highest amount, and Tarom Hashemi and Nemat cultivars stood third and fourth ranks. The three Khazar transgenic lines had the lowest amount for human health (Fig. 4). In terms of ecosystem quality, Tarom Molaii and Khazar cultivars showed the highest amount, and Tarom Hashemi and Nemat cultivars stood next ranks. The three Khazar transgenic lines had the least amount for ecosystem quality. In terms of climate change, resources and abiotic stock resources, Tarom Molaii and Khazar cultivars had the highest amount. The lowest amount of these three impact categories belonged to the three Khazar transgenic lines. The highest amount of biodiversity impact category belonged to three Khazar transgenic lines (Fig. 4).

4. Discussion

The investigation of the impacts of transgenic and non-transgenic crops on environment and human health is a controversial and considerable issue, which was dealt with in this research. The findings of this research showed that the in some issues non-transgenic rice cultivars had twice the adverse eco-impacts in many of the assessed impact categories (especially energy consumption and greenhouse gases emission due to the consumption of inputs and other agricultural operations) compared to transgenic rice cultivars. According to fossil fuels consumption for chemical pesticides production, it is considerable and important to consider life cycle assessment. In fact, the main reason for the differences in input energies and greenhouse gases emission between transgenic and non-transgenic rice cultivars was avoiding the use of chemical pesticides for fighting rice stem borer (*Chilo suppressalis* Walker) in transgenic cultivars, which led to a decrease in consuming the inputs and the energy of the sectors of pesticides, human force in agricultural operations, tools, machinery and fuel. In fact, these inputs are consumed without observing ecological issues in the production of non-transgenic cultivars. As well, different paddy yields in seven genotypes effect on this result. In this regard, other researchers have stated that the greenhouse gases' emission happens during different agricultural operations either directly through consuming fossil fuels during agricultural operations (planting to harvesting), or indirectly during the production and transfer of the field's needed inputs (herbicides, pesticides and chemical fertilizers) (Wood and Cowie, 2004). It was also stated in another study that agricultural and non-agricultural activities (the production and transfer of fertilizers and pesticides) in rice production play roles in global warming by producing 80–98 and 16–91 kg CO_2 eq per hectare, respectively (Pathak and Wassmann, 2007).

In terms of carcinogens and non-carcinogens pollutants transgenic lines had lower amount compared to non-transgenic cultivars. However, respiratory inorganics and respiratory organics impact categories in transgenic and non-transgenic cultivars were not significant. Tarom Molaii cultivar had more respiratory organics and respiratory inorganics than Tarom Hashemi because paddy yield of Tarom Molaii was less than that of Tarom Hashemi. The reason for higher energy consumption and global warming in non-transgenic cultivars can be attribute to their high dependence on inputs and higher energy consumption for production, and these inputs are used without any regard to environmental issues. Although most impact categories depend on genotype type and input and paddy field practice had not significant on impact categories. Different natural and human causes make global warming but global warming is mostly considered to be due to the increase in greenhouse gases emission because of human activities (Bare, 2011), which make a lot of changes in global climate patterns. In order to report the amount of the produced greenhouse gases, all the produced gases with the equivalent of CO_2, which states the global warming potential, are reported. In other research, GWP impact category in farming section was reported to be 119.5 kg CO_2 eq for wheat production (Wang et al., 2007), 1484–1847 kg CO_2 eq for rice in Rasht, Iran (Nabavi-Pelesaraei et al., 2014), 340 kg CO_2 eq for wheat in Marvdasht, Iran (Nabavi-Pelesaraei et al., 2016) and 381 kg CO_2 eq for wheat in Switzerland (Charles et al., 2006). The total energy consumed depending on the type of soil and field operations and production systems was reported to be 274–557 MJ in England (Tzilivakis et al., 2005), and 521 MJ for sugar beet production per ton in Japan (Koga, 2008).

Based on findings, it was observed that transgenic lines derived from Khazar have less emission of heavy metals into water, air and soil compared to their parent and the reason was less inputs. In fact, the amount of heavy metals into water and soil was calculated based on the annual estimation of these elements' deposit and also their entrance into soil through fertilizers, pesticides, seeds and deposit and their separation from soil by product harvest, leaching, and soil erosion. Also, in terms of energy demand, ecological footprint, greenhouse gas protocol and water footprint methods Tarom Molaii stood in first rank. Tarom Hashemi and Nemat cultivars stood second and third rank. The reason for the higher eco-toxicity impact in water and soil for producing non-transgenic rice cultivars compared to the transgenic ones can be attributed to the impact of spraying and using the active ingredient of pesticides during the plant's growth and development. Moreover, the consumption of fuel, machinery, production and transfer of these materials has toxicity impacts. The most important substances with acidification potential in ecosystems were SO_2 and nitrogen oxides, which are reproduced through consuming fossil fuels in the process of agricultural production. NH_3 caused by the consumption of chemical fertilizers in the field is also an important factor of acidification (Engstrom et al., 2009). These emissions cause acidification through the complex processes of atmospheric and chemical transfer, which damages the ecosystems, plants, and animal populations (Bare et al., 2003). In other research studies, the characterization index of the impact category of acidification was obtained to be 4 kg SO_2 eq (Wang et al., 2007). In another research in Chile the impact category of acidification for canola and sunflower production was calculated to be 19 and 23 kg SO_2 eq (Iriarte et al., 2010).

Considering the impact category of ozone layer depletion, Khazar cultivar had the highest amount and Nemat and Tarom Hashemi cultivars stood second rank. Tarom Molaii cultivar stood next rank in terms of ozone layer depletion. The three Khazar transgenic lines had the least ozone layer depletion, and all were at the same level. In terms of emission of biogenic CO_2 eq and CO_2 eq from land transformation, Tarom Molaii and Tarom Hashemi cultivars stood on top. There was no difference between Khazar cultivar and its transgenic lines in terms of emission of biogenic CO_2 eq and CO_2 eq from land transformation. Tarom Molaii and Tarom Hashemi cultivars got higher ranks than other cultivars. There was not significant in terms biogenic CO_2 eq and CO_2 eq from land transformation, between Khazar cultivar and its transgenic lines. For CO_2 uptake, Tarom Molaii cultivar stood on top, and Tarom Hashemi cultivar got the second rank. Regarding CO_2 uptake, no significant difference was observed among Khazar, Nemat and three transgenic lines derived from Khazar. Main reason for these results are insecticide consumption, genotype type, different paddy yield of genotypes and decreasing yield loss, dead heart and white heads by increasing transgenic cultivars resistance to stem borer. It is believed that emissions such as CFC and halogen gases damage the ozone layer in stratosphere (Bare et al., 2003). The ozone layer depletion can exert impacts such as skin cancer, molecular damages to materials, and damages to plants and animals, which are due to the increased penetration of ultraviolet rays (Bare et al., 2003). The rate of ozone layer formation in troposphere is determined by complicated chemical reactions which are influenced by the density of NO_x, volatile organic compounds and also temperature, sunlight, and convection currents. Recent findings showed that carbon monoxide and methane are also effective in the formation of ozone (Bare et al., 2003). Eutrophication is commonly dependent on the environmental impacts of releasing excessive amounts of nutrients, which changes the species combination of ecosystems and increases the production of biomass. This is followed by damaging consequences, such as decreased biodiversity, and chemical toxic compounds production for humans, livestock, and other mammals (Bare et al., 2003). Nemecek and Kagi (2007) reported the volume of eutrophication section leaching to be 0.59 kg N per a ton of sugar beet in Switzerland. Other researchers reported that the characterization index of eutrophication for producing canola and sunflowers was 7.2 and 9 kg PO_4 eq, respectively in Chile (Iriarte et al., 2010). Also, the characterization index of the acidification impact category for producing canola and sunflowers was estimated to be 19 and 23 kg PO_4 eq (Iriarte et al., 2010).

In some parts of the world, acid rain causes damage to plants and animals and increases soil acidification. One of the major sources of this impact on agriculture is the use of nitrogen fertilizers which releases NOx and NH_3 into the atmosphere. Since a lot of inputs are used in producing crops, the agricultural production system causes extensive environmental impacts (Brentrup et al., 2004b). In the study on rice in China, it was observed that for depleting the fossil resources for fossil fuel consumption was 106 MJ per ton and its final eco-index was obtained to be 0.008 (Wang et al., 2010). The finding of other researchers revealed that for producing a ton of canola in Turkey, 25.63 L diesel fuel (Unakitan et al., 2010), for a ton of soybean 87.78 L fuel (Ramedani et al., 2011), and for producing a ton of rice in Guilan 25.08 L diesel fuel (Pishgar-Komleh et al., 2011) were consumed. In a study on rice in China, it was found out that the water consumption of rice production was 379 cm per ton, and the final index was obtained 0.14 for the reduction of water resources (Wang et al., 2010). For the reduction of fossil resources, the fossil fuel consumption was 106 MJ per a ton rice production, and its final eco-index was obtained to be 0.008. In another study in north China, the final index for the reduction of fossil resources was obtained 0.02 for a ton of wheat production and 0.009 for a ton of corn production (Wang and Wu, 2009). Besides, in another study in Germany it was observed that in producing a ton of wheat, acidification and global warming were the main environmental impacts (Brentrup et al., 2004a). For wheat, energy depletion with the final index of 0.14 and acidification with 0.13 were the most important eco-indices (Wang et al., 2007). For producing sunflowers and canola, the highest environmental impacts were reported to be global warming and eutrophication (Iriarte et al., 2010). Photochemical oxidation potential (smog) is mainly due to the formation of ozone at the ground-level, which is itself influenced by the reactions between nitrogen oxides and volatile organic compounds in sunlight (Bare, 2011).

Dastan et al. (2016), with a life cycle assessment of conservation, conventional and improved system concluded that only CO_2 emissions showed high amounts as N_2O emission ranked second. Moreover, the conventional production system showed the same values for heavy metal emission in water (cadmium, copper, zinc, lead and chromium). However, the emission of all heavy metal in water for the conservation system was much lower than in the other two systems (Dastan et al., 2016). In the conservation production system, the emission of all heavy metals in the soil except for lead (cadmium, copper, zinc, nickel, chromium and mercury) showed a negative value. In the conventional and intensive production system, the emission of copper, zinc and mercury showed negative amount in the soil (Dastan et al., 2016). Other researchers compared the group of transgenic and non-transgenic rice cultivars, which stated that in terms of heavy metal emission in water (cadmium, copper, zinc, lead and chromium), all rice cultivars were approximately equal, the main reason being equal inputs in this section (Dastan et al., 2017).

5. Conclusion

In this research, life cycle assessment was done in the process of transgenic and non-transgenic rice cultivars production in order to estimate the impacts on human health and ecosystem. All indices of pollutants emissions were investigated through different methods. Results showed that decreased consumption of pesticides in transgenic cultivars had led to less use of human force, machinery and fuel, the result of which was a decrease in energy consumption, greenhouse gases emission and global warming potential. Furthermore, the highest emission of carcinogens, non-carcinogens, ionizing radiation, aquatic eco-toxicity, terrestrial eco-toxicity, terrestrial acid/nutri, aquatic acidification, aquatic eutrophication, non-renewable energy consumption, global warming and climate change were observed in the cultivars Khazar and Tarom Molaii. Conversely, respiratory inorganics, respiratory organics, terrestrial acid/nutri and aquatic acidification in GM and non-GM cultivars were not significant.

The heavy metals emitted in air (Pb, Cd, Zn and Hg) and water (Cr, Zn, Cu, CD, Hg, Pb and Ni) were less in transgenic lines than in non-transgenic cultivars. But, pollutants emitted of soil (nitrate, metals and pesticide) in transgenic lines derived from Khazar were less than their non-transgenic parent. The highest pesticide emission belonged to Tarom Molaii, and Tarom Hashemi and Khazar stood second and third ranks. For methane and lead emission, Tarom Molaii and Tarom Hashemi were first and second ranks. Methane emission in Khazar and its transgenic lines was equal. Fossil fuel depletion was statistically equal in Khazar cultivar and its three transgenic lines. The emission of fossil CO_2 eq and CO_2 uptake was equal in Khazar cultivar and its transgenic lines. In terms of emission of biogenic CO_2 eq, Tarom Molaii and Tarom Hashemi cultivars stood on top. In terms of human health based on DALY, no difference was observed among the cultivars. Regarding ecosystem quality, Tarom Molaii cultivar had the least amount, and Tarom Hashemi cultivar had the most. Three Khazar transgenic lines had the least amount for ecosystem quality. Therefore, most investigated impact categories were obtained high for Tarom Molaii and Khazar and less for three transgenic lines. According to the findings of this research, it can be claimed that adverse impacts on human health and ecosystem are related to pesticides usage, and this issue is ecologically important and is closer to sustainable development. Although the researchers in this study considered the consumption of pesticides for non-transgenic cultivars according to scientific standards, traditional farmers applied pesticides for producing rice two times or several times more amount. As a result, according to the findings of this research, it is concluded that the amount of the environmental pollutants emission is directly related to the consumption of inputs and method of field management, based on which the least amounts of these indices were obtained in the production of transgenic cultivars. Therefore, this issue is environmentally and ecologically significant and is closer to sustainable agricultural philosophy.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

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